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OBSERVATIONS BASED ON SYPHILIS-SEROLOGICALLY STUDIED MATERIAL

WITH SPECIAL REFERENCE TO THE POSITION OF THE CARDIOLIPIN AND SITOLIPIN ANTIGENS IN SYPHILIS REACTIONS AND TO THE INCIDENCE OF SEROPOSITIVE SYPHILIS IN TURKU AND PORI COUNTY (FINLAND) AND TO THE INCIDENCE OF CERTAIN DISEASES AMONG HOSPITAL PATIENTS WITH SEROPOSITIVE SYPHILIS

BY

LENNART KALLIOMÄKI

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Preface

The subject of the present investigation was suggested to me by Professor Eero Mustakallio, M.D., Chief of the Turku University Department for Bacteriology and Serology. He has provided me with the necessary investigation facilities and made the material of the laboratory under his direction available to me. The investigation has been carried out from beginning to end under his inspiring supervision.

Professor T. E. Olin, M.D. and Docent Risto Pätiälä, M.D. have followed my work with interest throughout, encouraged me in difficulties and given me invaluable advice on questions of venerology.

The Medical Professors of the Turku University Clinics, the Medical Director of the Pori General Hospital and all my colleagues in the Turku and Pori County have readily placed their patient materials at my disposal. Without this support it would have been impossible for me to do this work.

Dr. B. Sourander, Medical Officer of Health of Turku and Pori County has kindly permitted me to use the archives of his office and also assisted me in my work in other ways.

Without the helpful attitude of the directors of the Turku and Pori County prisons it would not have been possible to include prisoner material in my investigation.

My devoted and conscientious technical assistant throughout the work was Miss Ulla Härmälä. In the statistical treatment of my materials I have been guided by Mr. E. Arenti.

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were suggested by Mrs. Hilkka Kontiopää, M.A. (Helsinki) and Mrs. Joan Bramwell, M.A. (Oxon).

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Turku, January 1953.

Lennart Kalliomäki
Med.Lic.

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I.

Introduction and the Problems

Ever since WASSERMANN, NEISSER and BRUCK in 1906 stated that the aqueous extract of syphilitic tissue when added to syphilitic serum inhibited the fixation of the complement in the hemolytic system, serologic examination has played a most important part in the diagnosis of syphilis. In the following year LANDSTEINER, MÜLLER and PÖTZL used with great success the alcoholic extract of normal tissue instead of the aqueous extract of syphilitic tissue. In 1910 BROWNING, CRUICKSHANK and M'KENZIE observed that the Wassermann reaction, as this test was called, became more sensitive when cholesterol was added to the tissue extract. The very intensive research work which rapidly developed around these fundamental investigations, in a short time made the Wassermann reaction into a certain and clinically valuable serological test which, entirely unchanged in its essentials, has been successfully used for nearly half a century. The Wassermann modification which is used in Finland is chiefly based on the results of the investigations made by STRENG and MURTO (1913). Since then, a large number of investigations on this reaction have been carried out in Finland, all of which indicate, as ANTTONEN (1945) states when listing them, that the modification of the WR (Wassermann reaction) in use here is not too sensitive, as the number of nonspecific reactions must be considered low.

On the other hand, as compared with the development of the complement fixation reaction, the precipitation reaction for the serological diagnosis of syphilis has been much more hesitantly adopted. Already in 1907 MICHAELIS observed that flocculation occurred when syphilitic serum was added to the WR antigen; the precipitation, however, was not constant. At about the same

time JACOBSTAHL studied microscopically the mixture of this antigen and syphilitic serum and observed coarseness as compared with the normal serum. BOAS also claims to have studied this, but reports that he considered the phenomenon unsuitable for practical purposes because of the difficulty in evaluation (BOAS and PONTOPIPPIDAN 1921). The reactions of Fornet and Schereschewsky, Porges and Meyer, Elias, Neubauer, Porges and Salomon, Herman-Perutz, Klausner and Bruck for example have merely historical value as preliminary stages of the flocculation reactions. All these reactions were widely studied but none of them proved suitable for use because of their unreliability, as BOAS and PONTOPIPPIDAN (1921) state in discussing them. The first really serviceable precipitation reaction was that presented by MEINICKE in 1917, which in the form later modified by its developer is known as MEINICKE's third reaction. In the following year SACHS and GEORGI presented their own reaction using inactive serum, 0.85 per cent saline, and heart muscle extract. This reaction found a wide application and was introduced into Finland by MURTO (1920), who considered it a good reaction fully comparable with the WR, its cheapness being, in his opinion a great advantage over the WR. KAHN presented his reaction in 1923, and being simple and rapid the reaction quickly found wide acceptance and at present is probably the most frequently used precipitation reaction. In Finland its value and importance were brought into general knowledge by the investigations of STRENG, SIEVERS and VUORI (1934). Reference to MEINICKE's second clarification reaction (MCR II) presented in 1932, completes this survey of the most important developments up to recent years in the precipitation reactions used in syphilis diagnosis. These developments are covered by a literature comprising hundreds or perhaps thousands of publications, and innumerable precipitation modifications have been presented, but to deal with all of them would be irrelevant to the topic, and would indeed, be hardly feasible.

Further notable progress took place in the serological methods of syphilis diagnosis in 1941, when PANGBORN reported having isolated a new phospholipid from beef heart muscle, the structure of which she was also able to explain (1947). It has been generally known that the impurities in the heart muscle extracts employed have been one of the causes of false positive reactions. PANGBORN's

report was therefore received with enthusiasm. Cardiolipin — this name was given by PANGBORN to the phospholipid detected by her — has been the subject of active research work for several years. In the Finnish literature it was first dealt with by KALLIOMÄKI (1950). The adoption as a syphilis antigen of the substance called sitolipin, which UROMA and TUOMIOJA (1950) isolated from the wheat embryo, must also be considered remarkable. As it seems that throughout the world there is a tendency to pass over to the use of these antigens which are as pure as possible, and since experience, especially in regard to sitolipin, is so far rather meager — at least information is rarely found in the literature — one of the themes in this investigation is the position and value of cardiolipin and sitolipin as syphilis antigens in comparison with the earlier antigens employed.

Ever since the Middle Ages syphilis has been very prevalent and the destruction caused by it has been immeasurable. Every nation must, in my opinion, always know as definitely as possible of the part syphilis is playing among its people. Therefore, investigations on the incidence of syphilis are always valuable, the more so as its incidence varies from year to year. Because of this, another main topic of this work is the investigation, on the basis of the available seropositive syphilis series, of the incidence of seropositive syphilis in our laboratory series.

The literature on specific syphilitic diseases is very extensive, whereas few references only are found to the incidence of common nonspecific diseases among syphilities. With this in mind I have chosen as the third theme of my work the incidence of different diseases in the hospital patients included above mentioned seropositive syphilis series.

The objects of my work, which is concentrated around the three main themes mentioned above, are summarized in the following questions:

1. What has been the average sensitivity of the three seroreactions for syphilis — the WR, the WR sensitized with cholesterol, and the Kahn reaction — in the years 1946 — 50 in the Department of Serology and Bacteriology, Turku University, and what has been their behaviour in the various years?

2. What is the position of cardiolipin and sitolipin as syphilis antigens in the precipitation and complement fixation reactions as

compared with the three reactions earlier employed by us, with Meinicke's second clarification reaction (MCR II) and with the Berger reaction (a syphilis reaction carried out as a slide test, in which the Kahn extract sensitized with Victoria blue is employed as the antigen)? The two last mentioned reactions have been included in order to widen the sensitivity scale of the comparative reactions.

3. What, on the basis of the seropositive syphilis series in 1946 — 50 and partly also in 1951, is the incidence of positive syphilis in the area served by our laboratory, which is mainly tricted to the County of Turku and Pori, and what is this incidence among the pregnant women, convicts, alcoholics and inmates of communal homes included in the material?

4. What on the basis of the seropositive syphilis series in 1946 — 50 and partly also in 1951 is the incidence of other than specific diseases among the seropositive syphilities, and is there any difference as compared with the incidence of various diseases among nonsyphilitic persons in hospitals during the corresponding period?

II.

Material

The seropositive syphilis series, the various aspects of which will be examined in the light of the foregoing questions, originated in the following serogically tested materials:

— A series of 126,344 blood specimens examined in the period 1946 — 50 in the Department of Bacteriology and Serology, Turku University. The Kahn with was made reaction each specimen; with 79,719 specimens the Wassermann reaction and the Wassermann reaction sensitized with cholesterol were also made.

— A series of 7,928 blood specimens examined in 1951. In addition to the three routine reactions mentioned above, two cardiolipin slide tests and one sitolipin slide test were carried out with each specimen. Meinicke's clarification test was also performed with 4,260 specimens and the Berger slide test with 4,414 specimens. All eight reactions were carried out with 3,014 specimens.

— A series comprising 5,116 blood specimens examined in 1951. With each specimen the routine reactions were made — the Wassermann with and without cholesterol and the Kahn — and in addition the Wassermann reaction with cardiolipin and sitolipin as antigens. The Wassermann reaction using these four different antigens was also made with 238 cerebrospinal fluid specimens. All the serologically examined specimens were contributed by hospitals, physicians, consultation centers, prisons and other institutions, chiefly in the County of Turku and Pori.

III.

Serology, with Special Reference to the Cardiolipin and Sitolipin Antigens

SOME FEATURES OF CERTAIN CURRENT PROBLEMS IN THE SEROLOGIC DIAGNOSIS OF SYPHILIS

Syphilis Antibodies

When speaking of the syphilis antibodies we deal with theories only. The textbooks mention the spirochaetal-antibody, auto-antibody, spirochaetal-antibody + auto-antibody, and the labilisation theories. None of these has as yet been conclusively shown to be correct, neither has any of them been proved false. Recent investigations however, have been able to present certain new features. When EAGLE and HOGAN (1940) adsorbed syphilitic serum with heart muscle lipids, the seroreactions performed with the lipoidal antigens after the adsorbtion were negative, while the serum continued to react with the spirochaetal suspension antigen. The authors ascribed this circumstance to the presence of antilipoidal and antitreponemal antigens. Using an adsorbtion system of a similar kind D'ALESSANDRO (1946) reported that he could demonstrate the existence of three different antibodies. These are the antilipoidal antibody, the thermostabile antitreponemal antibody and the thermolabile antitreponemal antibody. Of the treponema-specific antibiodies the thermolabile antibody is of the nature of a protein, a thermostabile polysaccharide and haptene (D,ALESSANDRO, ODDO, COMES and DARDANONI 1949). PUCCINELLI and ODDO (1946) confirmed the investigations of their compatriot. In primary syphilis, according to D'ALESSANDRO (1946) the antitreponemal antibodies predominate, while in prenatal syphilis the antilipoidal antibodies predominate. When studying the presence of these three antibodies PUCCINELLI and REZZI (1949) came to the following result:

1) If all the three antibodies were similarly present in the serum, it indicated activity of the disease; 2) The occurrence of the antilipoidal antigen alone could be due to congenital syphilis or to a nonspecific reaction; 3) If the antitreponemal but not the antilipoidal antibody was present, the disease was in the preserological stage; 4) The absence of all the reagins was of significance in making the prognosis of the disease; 5) The significance of the thermostabile antitreponemal antibody was greater than that of the thermolabile antibody, as the latter had a tendency to disappear from the sera.

According to the auto-antibody theory the organism forms antibodies against its own tissue lipids released by syphilis (SACHS, KLOPSTOCK and WEIL 1925). BECK (1939), however, has been able to prove that the alcoholic extract of a cultivated spirochaetal emulsion contains lipids similar to those in the corresponding extract of the tissues. Thus the antilipoidal antibodies might also be of specific origin. All the investigations on the syphilis antibodies have had the drawback that they have employed various strains of treponema, which from experience must be regarded as avirulent and which serologically are not identical but may be divided into different groups. However, all of them were originally cultivated from specific lesions and were believed to be true *Treponema pallidum* (EAGLE and GERMUTH 1948, KHAN, NELSON and TURNER 1951).

NELSON (1948) has succeeded in keeping alive Nichols's strain of *T. pallidum*, from which a laboratory worker acquired a laboratory infection and which accordingly must probably be considered a human pathogen. If this virulent strain of Nichols's was incubated for 16 hours in + 35° C together with syphilitic serum, it lost its mobility. This so called immobilization reaction was reported in 1949 by NELSON and MAYER. After adsorption of syphilitic serum with the lipoidal antigen they continued to obtain a positive reaction. This they considered to be a proof of the presence of some other reagent than the one reacting with lipoidal antigen. The infective power of the strain employed in the reaction disappears somewhat earlier than its mobility (THOMPSON, GREENBERG and MAGNUSON 1950). The authors are nevertheless of the opinion that both phenomena are caused by the same antibody. ARCHAMBAULT (1950) also observed that the virulence of the strain used in the immobilization reaction decreases.

Of 63 sera, which yielded evidently biologically false positive seroreactions, 56 gave a negative immobilization reaction, 6 a positive and 1 a doubtful reaction. In 126 treated, definitely diagnosed cases of syphilis this reaction was positive in 96,8 %, and 16 out of 67 probable cases of latent syphilis gave a negative immobilization reaction (MOHR, MOORE, NELSON and HILL 1950). According to the authors it is uncertain whether the antibodies concerned in the reaction will disappear by treatment. DESBORDES and DELAUNEY (1950), again, are of the opinion that the reaction should be made in untreated cases only. In the secondary stage of syphilis it is always positive, according to them. KHAN, NELSON and TURNER (1951) studied various species of treponema by means of the immobilization reaction. A definite cross-reaction was seen between the *T. pertenua, T. cuniculi* and *T. pallidum* (Nichols's strain). Rabbit serum immunized with Reiter's, Kazan's and S-26 strains did not yield an immobilization reaction with *T. pallidum, pertenua* and *cuniculi.* In quantitative tests there was a difference in titer between *T. cuniculi* on the one hand and *T. pertenua* and *pallidum* on the other hand. The two last mentioned also gave a similar quantitative reaction. The authors also observed that the spirochaetes taken from the testicles of the rabbit immediately after the development of syphilitic orchitis were more sensitive than normally in the immobilization reaction (sensitization *in vivo*). The measurement of the immobilization antibodies did not give any indication as to the ability of the rabbit to withstand infections caused by three different strains of *T. pallidum.* Neither could any differences be observed between these three strains in the immobilization reaction made *in vitro* (MAGNUSON, THOMPSON and MCLEOD 1951). In connection with the immobilization reaction is should be remembered that normal human serum contains antibodies against some spirochaetes of the mouth (KOLMER, KAST and LYNCH 1941) and that the sera of healthy human begins agglutinate Reiter's strain in dilutions of 1/40 — 1/80 (KRECH 1951). With this may perhaps be connected the observation of THOMPSON, MAGNUSON and HILL (1951) that by increasing the concentration of the guinea-pig serum the sensitivity of this test is increased. The authors hold the opinion that the reaction is important in the study of immunity and the biologically false positive reaction, but that it is not sufficiently reliable to be a routine test for syphilis.

The supposition that the syphilis antibody might be a bivalent cation like Ca and Mg requires additional investigation (PIERCE and BREAZEALE 1942, MALTANER and D'ALMEIDA 1948, REIN and KOSTANT 1949). The postnatal complement fixation test for the child of a syphilitic mother is more often positive than the flocculation test (MOORE 1947). REIN and KOSTANT (1949) combined this observation and that made by WIENER (1948) regarding the existence of two Rh-antibodies — a bivalent one, which does not pass through a healthy placenta (agglutinin), and a univalent one (glutin) which passes through the placenta, causing the disease picture of erythroblastosis foetalis. According to these authors, this may also be the case with the syphilis reagins, and the reagins involved in the complement fixation test may be univalent and those taking part in the flocculation test bivalent.

The fact that the antibodies are contained in the globulin fraction of the serum is generally accepted, and BJØRNEBOE (1941) has even been able to demonstrate that all the gloubulins of a hyperimmunized rabbit are replaced by antibody gloubulins. LANDSTEINER and MÜLLER (1908) observed that every healthy and syphilitic serum yielded, when carbonic acid was added, a precipitate which gave a positive Wassermann reaction in saline solution. The same result was reached also by BAUER and HIRSCH (1910) and by GROSZ and VOLK (1910). These investigators were of the opinion that the Wassermann reagins move with the globulin fraction of the serum. FRIEDEMANN (1910) stated on the basis of his studies that these reagins follow the euglobulin fraction and that they are anticomplementary in syphilitic serum only, because in normal serum the albumin fraction acts as an antagonist and prevents the reaction. The same result was reached by SCHMIDT in the following year. BAUMGÄRTEL (1922) maintains that the syphilis reagins arise from the serum globulins due to syphilitic infection. He also assumes that this is a lipid globulin. Many investigators have been able to demonstrate increased serum globulin levels in active syphilis (*e.g.*, COOPER 1945, VERHAGEN 1947, PEREZ SANDOVAL 1950, KALLIOMÄKI 1951). As a result of treatment the increased globulin values revert to normal (COOPER 1945, PEREZ SANDOVAL 1950). The albumin levels are frequently somewhat reduced at the expense of the globulins, and thus the proportion between the albumins and the globulins is decreased, according to PEREZ SANDOVAL

(1950), by almost 100 %. The albumin fraction in syphilitic sera is serologically inactive (*e.g.*, NEURATH, VOLKIN, ERICKSON, CRAIG, PUTNAM and COOPER 1947).

The ultracentrifuge and electrophoresis were also adopted soon after their invention for the study of syphilis reagins. According to KABAT (1946) the syphilis reagin consists of two different components, the sedimentation constants of which are 7 and 9 Svedberg. The electrophoretic mobility of the reagin was observed in his studies to be between the β and γ globulins, which finding had also been made by DAVIS, MOORE, KABAT and HARRIS in the previous year. 50 % of the serologically active euglobulin fraction belong to the γ globulins (ERICKSON, VOLKIN, CRAIG, COOPER and NEURATH 1947). In their investigations, the more rapidly moving globulins, *i.e.*, those nearer to the β globulin fraction, were also serologically more active than the slower γ globulins. When examining the decrease in globulins which takes place in syphilities after an injection of HgJ_2 (so called D'Amato's hemoclastic syphilis reaction), KALLIOMÄKI (1951) observed that the greatest decrease occurred in the α_1 and β globulin fractions. When studying the phenomenon he presents the supposition that the specific antitreponemal antibody is a lipoprotein, which moves with the α_1 or β globulin fraction or with both, whereas the antilipoidal antibody moves with the globulin fraction. COOPER, CRAIG and BEARD (1946) had earlier observed that in syphilitic sera the α_1 and γ globulin fractions were larger than normal. However, this does not fully agree with the generally accepted and proved fact that the antibodies move with the γ globulin fraction (BOYD 1947). Nevertheless there are exceptions to this general phenomenon. Thus antibodies against tuberculin proteins can be demonstrated which move with the α globulin fraction (SEIBERT and NELSON 1942). The diphtheria antitoxin of the horse, again, moves not only with the γ but also with the β globulin fraction (KEKWICK 1941, KEKWICK and RECORD 1941).

From the above survey based on the literature dealing with the syphilis antibodies we can only draw the conclusion that no theoretically tenable explanation of the question has as yet been found. Nevertheless it seems to be considered certain that several syphilis antibodies are concerned in the different serological reac-

tions and that at least a part of them are true antibodies acting against the Treponema pallidum.

False Positive Seroreactions for Syphilis

Nature and Occurrence of False Positive Reactions

False reactions have always been one of the most central problems in the serological diagnosis of syphilis. And they are likely to continue to be so in the future, for in a biological test we shall hardly ever be able to obtain results that are 100 % accurate ROEMER (1949) considers that the false positive reactions are due to one of the following two causes: 1) Primary alterations in the patient's serum caused by nonsyphilitic diseases or therapeutic measures; 2) Errors in connection with the taking of the blood sample and factors affecting the blood thereafter. MONTESTRUC, BLACHÉ and LABORDE (1951) use the following classification: 1) Positive reactions due to technical errors; 2) Syphiloid reactions (*e.g.* tropical ulcer, leptospirosis, filariasis, leishmaniasis, leprosy etc.); and 3) Biologically false positive reactions (false reactions occurring in diseases other than those mentioned above). The syphiloid reactions correspond to the "noncharacteristic reactions" of WITEBSKY and KRAH (1930) and the biologically false reactions to their "nonspecific reactions". According to these workers, the noncharacteristic reactions are true reactions due to antibody formation, such as are seen in spirochaetal and trypanosomal diseases and leprosy, but they also included in this group the false reactions occurring in endocarditis lenta, pneumonias, tuberculosis, scarlatina, diphtheria and mononucleosis infectiosa. The nonspecific reactions, according to them, are due to structural changes in the serum protein which are caused by some severe systemic disease and lead to false seroreactions for syphilis. Accordingly, the borderline between the two types of reaction presented by them is very diffuse.

DAVIS (1944) mentions the following three hypotheses by means of which attempts have been made to explain the biologically false reactions (he includes the "syphiloid reactions" in the biologically

false reactions): 1) They have been ascribed to the production of antibodies which are immunologically identical with the syphilis antibodies; 2) On the other hand, it has been suggested that the antibodies differ from the syphilis antibodies but are cross-reactive with the antigens employed; 3) It has also been suggested that this is not a true antibody-antigen reaction but rather a kind of nonspecific reaction between the antigen and a serum component of unknown origin.

False positive reactions are also seen in the case of entirely healthy human beings and animals (*e.g.*, EAGLE 1937, KRAG and LÖNNBERG 1938, MAASSEN 1948, MOORE 1949, DE GÓES 1950, DENECKE 1951). Of these workers EAGLE calculated that the frequency of such reactions in human beings is 1/5000 and MOORE 1/3000. The latter holds the opinion that the standard seroreactions are based upon a nonspecific antibody in the serum and that such nonspecific reagins are probably present in all human beings. Accordingly, one person out of every 3,000 would have so many of them that a false positive reaction was produced. DEGÓES (1950) ascribes the reaction to a biological reagin which is found in both man and animals. KOLMER, KAST and LYNCH (1941) state that normal human serum contains antibodies against certain spirochaetes of the mouth and that these may be the source of reactions of this kind. WIDELOCK, GONSHOREK and MARSDEN (1950) found in 2.1 % of 5,048 routine samples a factor which reacted with the acetone-soluble part of extracts of organs. They consider that this serum factor is partially responsible for the false results obtained in the Mazzini reaction. According to HARTMANN and SCHØNE (1943), false reactions of this kind may occur in the complement fixation test because a latent anticomplementary factor α in the extract used as the antigen is activated by a factor β in certain sera, thus producing the false reaction. Accordingly this would not be an antibody-antigen reaction. The flocculation reactions would be negative in such cases. However, it is difficult to exclude the possible role of syphilis in a positive seroreaction persisting in a clinically healthy person, and thus BRENES (1949), for example, is of the opinion that such a reaction is always due to syphilis.

PELS LEUSDEN (1950) suggests as the cause of the false positive syphilitic seroreactions occurring in different infectious diseases the possibility that due to the infection the homologic albumin

becomes heterologic and causes the formation of antibodies, which in turn produce the false positive reactions. BREDE (1951 *b*) also pays attention on the fact that false reactions appear especially in diseases and conditions where alterations in or decomposition of tissues take place, as in pulmonary tuberculosis, gastric and intestinal ulcers, tumors, typhoid fever, pneumonias, malaria and endocarditis.

When examining women in whom transient false positive seroreactions for syphilis occurred during menstruation WILDFÜHR (1949) observed that most of them had some kind of allergic disposition either in themselves or in the family. He suggests the possibility that allergy might also play a rôle in the origin of false positive results.

The interest of investigators has also been aroused by the possibly demonstrable clinico-chemical properties of the serum reagins involved in these primary false positive reactions arising from the original nature of the serum. ROSENBERG (1945) holds the opinion that the nonspecific factor and the syphilis reagin are in different serum fractions. He came to this conclusion when studying the false positive results for malaria. COOPER (1945) did not find in all the sera leading to false positive results the increased globulin values which he found in active syphilis. On the other hand, VERHAGEN (1947) stated that false positive reactions are found mostly in diseases in which there is γ-hyperglobulinaemia. KALLIOMÄKI (1951) presented the assumption that it is the antilipoidal antibodies that move with the γ globulin fraction and are the cause of the false reactions. No differences have been observed in the electrophoretic mobility of the false and specifically positive sera (*e.g.*, MALMGREN 1946, NEURATH, VOLKIN, ERICKSON, CRAIG, PUTNAM and COOPER 1947). In the investigations of SIEVERS (1948) the false anticomplementary power of the serum was associated with altered thymol values and a decreased mercuric chloride test; it had no correlation to the serum phosphatase, citric acid and the icterus index. According to PACKALÉN (1948 *b*) the factor causing nonspecific increased antistreptolysin titers moves with the β and α globulins or with the same fractions as cholesterol. He therefore suggests (1948 *a*) that it might be possible to eliminate the false positive serological syphilis reactions by removing the lipids from the serum. BREDE (1951 *b*) obtained for healthy sera the iodine number of 4 — 7

mg/cc; in the whole series of 200 syphilities none had below 8 mg/cc. On the other hand, in sera giving false positive reactions it was higher due to the increased lipoproteins.

The chances of false positive results caused by technical reasons arise immediately after the drawing of the blood sample from the blood vessels. These possibilities may be grouped as follows: 1) False results caused by factors affecting the serum; 2) False results caused by defects in the reagents (antigens, hemolytic system, electrolytes used for the reaction; 3) False results caused by errors in the technical performance of the reaction; 4) Clerical errors.

Among the disturbing factors affecting the serum are impurities in the test tubes and vessels. Acids and ether may give rise to false positive results (SIEVERS 1939). Alkalis and alcohol may cause a positive serum to give a false negative reaction (VONDERLEHR (1936, SIEVERS 1939); this also true of salvarsan (VONDERLEHR 1936). Due to acids contained in them, corks and water may cause false positive results (DENECKE 1951). It was observed by SNELLMAN (1924) that sera infected with *B. subtilis* and *St. aureus* became self-inhibitive in the Wassermann reaction in 4—6 days. In sera infected with *B. subtilis* TAKANO (1935) obtained positive results before the occurrence of self-inhibition. It is possible however, that his *B. subtilis* was *B. cereus,* as the differences between the two are very slight (SIEVERS 1939). PROCHAZKA (1930) also obtained positive reactions with *B. subtilis.* The most regular cause of false positive reactions in these bacterial infections are *B. cereus* (PROCHAZKA 1930, SIEVERS 1939, AMINOFF 1939) and *B. mycoides* (PROCHAZKA 1930, AMINOFF 1939). Bacterial infections of this kind and probably also colloid-chemical changes occurring in the serum when left standing, etc., indicate the necessity of examining the serum as soon as possible after taking of the blood specimen (*e.g.,* FORSSMAN 1922, GABRIEL and KAIL 1949, PENTTINEN and HELLE 1949 *a*). It is also well to remember that such an effective storage medium as merthiolate does not in itself influence the results (REIN and BOSSAK 1946 *b*).

With regard to the defects in the reagents used for the reactions, the following may be mentioned: the antigen may be impure and, for example, possess already in itself an anticomplementary power; there may be faults in the hemolytic system used in the complement fixation, as instance in the titrations of the amboceptor and

the complement or in the amboceptor or complement itself; in flocculation reactions the preparation of the antigen emulsion with the electrolytic solution may prove a failure and the electrolytic solution be impure or of a wrong concentration (EAGLE 1937, PENTTINEN 1946). If, for example, in reactions in which the final result is the precipitation of the lipids, the antigen employed is an extract containing an excess of lipids that precipitate under the influence of the electrolytes, false results may follow (MURTO 1921).

In the technical performance of the reaction errors may occur at every stage. Inactivation may occur at a wrong temperature. Errors in measurement may be made in pipetting. PENTTINEN and HELLE (1949 *a*) have compared the results obtained by automatic and manual pipetting. There were less technical errors in the reaction series when automatic pipetting was used, since it gives stable results and prevents the addition of excessive or deficient amounts of electrolytes, which are liable to give false results in either direction. On the other hand, it should be held in mind that storage and after-inactivation may produce changes in the reaction which are not to be considered signs of false positivity (PENTTINEN and HELLE 1949 *a*).

Due to human fallibility, clerical errors could not be totally eliminated even if numerous controls were employed. In the series of 150,000 reactions made by KRAG and LÖNNBERG (1938) the incidence of such errors was estimated at 1/15,000.

An example to show the extent to which false positive results caused by these technical errors appears in Finnish material, and may be seen in the studies of PENTTINEN (1946) and PÄTIÄLÄ (1950). PENTTINEN studied 18,090 blood specimens from pregnant women. In this series there were 92 false positive results, 70 of which (76 %) proved to be technical errors. PÄTIÄLÄ's investigation covered 1,878 school pupils aged 10 — 14 years. Using the whole material as the basis of comparison he found the percentage of technical errors to be 0.32 for the Wassermann reaction, 0.21 for the Wassermann reaction sensitized with cholesterol, and 0.32 for the Kahn reaction.

Biologically false positive seroreactions for syphilis have been reported for numerous different diseases and conditions. As THOMAS (1949) says, "the list of conditions accused of producing false positive STS (serologic tests for syphilis) grows each year, and no

doubt it will continue to increase with our present sensitive tests". He lists the following diseases and conditions in which such biologically false reactions are often observed: leprosy, malaria, lupus, erythematosus, typhoid fever, mononucleosis infectiosa, rat-bite fever, Weil's disease, brucellosis, smallpox vaccination, lymphogranuloma venerum, upper respiratory tract infections, leuchaemias and Banti's disease. BÜTTGENBACH (1951) enumerates the following diseases and conditions: febrile conditions, anginas, mononucleosis infectiosa, scarlatina, malaria, typhoid fever, tuberculosis, leprosy, spotted and recurrent fevers, tumors (especially far advanced carcinoma), severe staphylococcic infections, balanitis gangraenosa, endocarditis lenta, leuchaemias, mycosis fungoides, ulcerations caused by fusiform bacilli and spirilli, subacute pneumonia and bronchopneumonia, serum treatment for diphtheria, digitalis therapy, menstruation, pregnancy, parturition, and narcosis.

As leprosy and malaria are diseases which are considered rare in the northern countries, the various infections of the respiratory tract are here the most important causes of biologically false positive reactions (KRAG and LÖNNBERG 1938, BORGEN 1948, WIINGAARD 1948). A more comprehensive list of literature on this subject in Northern Europe is given by PENTTINEN (1946). Among the diseases of the respiratory tract yielding false positive results WIINGAARD (1948) mentioned especially laryngobronchitis, bronchopneumonia and severe diseases of the nasal mucosa. False positive results are also found relatively often in primary atypical pneumonia (*e.g.*, FLORMAN and WEISS 1945, SAVONEN 1948). In the series of FLORMAN and WEISS comprising 68 such cases of pneumonia, transient positive seroreactions for syphilis developed in 17 % of the cases. The concept of "Wassermann-positive lung infiltrate" was introduced into the literature by HEGGLIN and GRUMBACH (1941) to indicate pneumonia with a subacute course in which the reactions are positive for syphilis. In WIINGAARD's (1948) series of 721 false positive sera, herpes genitalis and mononucleosis infectiosa were also well represented. In the material of KRAG and LÖNNBERG (1938), which comprised 53 strongly positive false reactions in a series of 157,500 sera, 5 were from herpes genitalis patients. On the other hand, BLUM and COLLART (1949) and BÜTTGENBACH (1951) emphasize that syphilis should be suspected in every alteration in the genitals.

A group of its own among the diseases of the lungs is formed by pulmonary tuberculosis, which many investigators have found to yield nonspecific syphilis reactions. MÜLLER (1913) examined 400 cases of severe pulmonary tuberculosis, 3 of which gave a definitely positive and 7 a weakly positive Wassermann reaction. Where there was no question of actual syphilis. However, he was of the opinion that in tuberculosis of the lungs a positive Wassermann is somewhat infrequent (< 1 %). STRYJECKI (1938) reported that he obtained a nonspecific positive reaction from the sera of tuberculotics in 9.3 % of the cases. Out of 99 tuberculotics examined by STRENG, SIEVERS and VUORI (1934) 2 yielded a false seroreaction for syphilis. In ANTTONEN's (1945) series of 45 tuberculotics 7 gave positive reactions, 3 of which proved to be false. JENTGENS and BREDE (1949) observed the syphilis reactions during Tb I/168 treatment in 70 cases and found 27 definitely and 4 doubtfully positive Kahn tests. They hold the opinion that these reactions which had developed during the treatment had some connection with the lipids released from the tubercle bacillus. However, many investigators are of the opinion that the incidence of false positive reactions in tuberculosis is no greater that in the control series (*e.g.*, DOWNS 1922, EAGLE 1937, BRAND 1949, ROTTMAN 1949, CASELITZ 1951). For instance, in BRAND's series of 351 tuberculotics there were only 4 cases with definite false positive seroreactions.

As to mononucleosis infectiosa, according to some investigators no more than the normal number of false positive results are found in it (GRAETZ and TETZNER, TZANCK, SIDI and BELLEMARE 1947, CASELITZ 1951). The material of the first mentioned of these comprised 80 patients suffering from this disease; of these none developed positive seroreactions of syphilis. TZANCK, SIDI and BELLEMARE again studied 3,000 Paul-Bunnel reactions, on which sera the Wassermann was also performed. In this material 2 positive Wassermann developed.

The question of the appearance of false positive results in the pregnant, the vaccinated and blood donors is of practical significance, because these may form a considerable part of those investigated in the serologic routine diagnostics.

Regarding the false positive results appearing during pregnancy reference is made to the comprehensive study by PENTTINEN (1946) on this subject. His material comprised 18,090 pregnant women of

whom 451 had either the Wassermann or the Kahn or both positive. Of the positive results 25.1 % were false. Only in 14 cases could the false reaction be considered to be due to pregnancy in the first place. The author is of the opinion that during pregnancy false positive reactions may develop, but that these are no more common than in control materials.

The most important cause of false positive results among vaccinations is small-pox vaccination. Regarding these reactions most of the investigators have come to similar results. The reactions generally become positive in 10 — 15 days and turn negative in 3 months (*e.g.*, REIN and ELSBERG 1945, DELAVERGNE, WATRIN and CAUCHOIN 1949). SINGH (1947) obtained a frequency of 14.3 % for false positive results, the French investigators DELAVERGNE, WATRIN and CAUCHOIN maintained that small-pox vaccination may lead to false positive results in 18 % of the cases; according to them the flocculation reactions yield these positive results more often than the complement fixation reactions. The material of REIN and ELSBERG (1945) comprised 129 soldiers who, from among a large group of vaccinated, had had some vaccination reactions. False positive reactions developed in 44.9 % of them; those who had had the vaccination had a higher titer value in these reactions than those in whom the vaccinoid reaction developed. In their material also the smallest number of false positive results appeared in Kolmer's complement fixation. The study of BAY and SANKSTONE (1940) however, is among those that give the opposite results. They followed the seroreactions of 100 vaccinated, none of these developed a positive Kahn. Various kinds of vaccines may also produce false reactions. CARRILLO (1948) reports that intravenous antichancrous vaccine gave false positive results in 33 % of cases, intravenous antityphoid vaccine in 14 — 17 % of cases and antigonococcal vaccine in 8 % of cases.

STOKES, BOERNER, HITCHENS and NEMSER (1946) analyzed the serological condition of more than 200,000 blood donors. 489 of them had positive reactions, of these 59.5 % proved to be false positive reactions. BARNARD, REIN and DOAN (1946) are of the opinion that the minimum frequency number of false positive reactions developed in blood donors is 0.4 %. They also classified their material according to the of times they had given blood; it appeared that the largest number of positive results was among those who

were giving blood for the second time, those giving blood for the first and third time were on the same level, but at the fourth time a noticeable decrease was observed in the frequency of these positive results. TZANCK, SIDI and BELLEMARE (1947) calculated the frequency number of the false positive reactions appearing in blood donors as 1/1000.

The prevalence of hepatitis makes it advisable to give some figures on the incidence of false positive results caused by this. KUZELL and PUCCINELLI (1944) report having obtained false positive results in some stage of the disease in 20 % of those suffering from acute hepatitis. HAVENS, GAMBESCIA and KNOWLTON (1948) followed the course of the Kahn reaction in 388 hepatitis patients, some of whom were volunteers and all free from syphilis. A positive Kahn developed in 1.5 % and a doubtful positive reaction in 2.5 %. SIEVERS (1948) investigated 374 icteric sera, of these 28 caused inhibition in the Wassermann reaction; the anticomplementary ability of all these sera had increased. CASELITZ (1951) also reports having obtained false positive results in hepatitis and he mentions that the well-known clinician EPPINGER also holds the opinion that in hepatocellular diseases such false positive seroreactions of syphilis are obtained.

It is not my intention to consider more closely the innumerable other diseases, disease groups and various pathological and non-pathological conditions in which false seroreactions of syphilis have been found; this would involve a survey of almost the whole science of medicine. It may, however, be mentioned that in MÜLLER's (1913) material, comprising 5,000 diseases of various kinds, mainly severe, the frequency of false positive Wassermann reactions was 2.4 %. Moreover, it should be remembered that they may also occur familially due to some inflammatory disease or similar condition (*e.g.,* SMITH 1937, LINDAU 1939, REYN 1941) and that in different continents and even in different districts of one country their incidence may be different (MONTESTRUC, BLACHÉ and LABORDE 1951, STOUT and CUTLER 1951). It should also be mentioned that the true syphilis reagins in serum might nonspecifically impede other serological reactions as is the case, for example, in the serological pertussis diagnostics (KYRKLUND 1917).

On methods of distinguishing false positive reactions from specific reactions

In 1940 KAHN published his first "verification test" according to which, at the temperature of 0° C, the syphilitic serum does not yield a positive result in the Kahn reaction, whereas sera reacting biologically falsely do. The majority of the investigators who have discussed the practical value of this reaction are, however, of the opinion that it is not reliable (PENTTINEN 1946). KAHN (1943) also published a second method for the verification of false reactions. According to him the precipitates of the positive Kahn reactions disperse, if a false reaction is concerned, when 30 % saline solution is added. This, however, does not occur in the syphilitic reactions. PENTTINEN and HELLE (1949 *b*) investigated 3,424 positive sera by means of this method and arrived at the result that it is not of any great value in verifying false reactions, as the majority of the reactions even those in which the dispersion was most complete, were specific.

BROWN, KOLMER and LYNCH (1945) report success in inhibiting the false positive Kolmer, Kahn and Mazzini reactions in normal rabbits by adding 0.1 ml of N/3 NaOH to 1 ml of serum. With the same quantity they had also been able to inhibit the false reactions appearing in man in malaria, mononucleosis infectiosa and virus pneumonias; in leprosy this had succeeded more rarely, on the other hand the procedure also produced false negative results in syphilitic sera of low titer.

NEURATH, VOLKIN, ERICKSON, CRAIG, PUTNAM and COOPER (1947) observed that in syphilitic sera the total titer of the albumin and globulin fraction was equal to or smaller than the titer of the reaction made of the whole serum, whereas in biologically false positive sera the titer of the globulin fraction (fractions G I and II) was higher than the titer of the whole serum. Crude albumin fraction inhibits biologically false positive reactions in 50 %. Crystalline albumin alone does not inhibit. When this phenomenon was investigated in greater detail, it was observed that the most active inhibitor of biologically false reactions was fraction IV, which corresponds to the electrophoretically obtained albumin + α-globulin fractions. The analogous fractions of the calf and guinea pig were inactive in this sense. With cholesterol the inhibitor factor could

be totally adsorbed (VOLKIN, NEURATH, ERICKSON and CRAIG 1947). The inhibition phenomenon is dependent on the relationship of the inhibitor and the antigen (VOLKIN, NEURATH and CRAIG 1947). These investigators assume that in syphilitic serum the antibodies evidently have a stronger affinity with the antigen than with the inhibitor. Out of 409 biologically false positive sera 92 % yielded a biologic false positive type of reaction and 2 % a syphilitic type in this inhibition reaction. Out of 386 syphilis sera 95 % gave a syphilitic type and 2 % a biologically false positive type reaction (NEURATH, VOLKIN, CRAIG and ERICKSON 1947). These investigators also observed that in fresh untreated primary syphilis biologically false positive reactions were often obtained. VOLKIN (1949) holds the opinion that the inhibitor is a serum lecithin. Egg lecithin has no inhibiting effect, while heart muscle lecithin has. This inhibition reaction has aroused great hopes, but, evidently owing to the great technical difficulties connected with it, the reaction has nevertheless found no place in the routine work.

The idea that the albumin fraction might affect immunoglobulin in some inhibiting manner is not new as such. PICK (1901) advanced the theory that nonspecific serum proteins, especially albumins, have a promoting effect on the denaturation of immunoglobulins, and this supposition was experimentally proved by STRENG (1909). FRIEDEMANN (1910) observed that although the normal euglobulin fraction as such was anticomplementary, the albumin fraction nevertheless acted as antagonist in the whole serum, and thus the Wassermann reaction was not sufficient, however, and the whole serum thus became anticomplementary.

SIEVERS (1948) observed that if serum in which the Wassermann is negative but the flocculation reactions positive is added to a known positive serum, a higher titer is received than by dilution with saline solution. This "additive effect" could not be obtained in the sera of completely treated patients nor in sera giving false positive reactions. SIEVERS is of the opinion that this method might be of importance in the corroboration of the flocculation reactions.

Many investigators are of the opinion that Nelson's treponemal immobilizing test is specific to such an extent that with it biologically false positive reactions can be eliminated (*e.g.*, NELSON, ZHEUTLIN, DIESENDRUCK and AUSTIN 1950, MOHR, MOORE, NELSON and HILL 1950). It is to be regretted that its introduction is restric-

ted especially in smaller laboratories by the technical difficulties connected with it (Reyn 1951).

Brede (1951 *b*) uses iodometry to distinguish false positive results. In the sera giving false positive results there is according to him an increased iodine number, as has been mentioned previously.

Several different methods of provocation have been applied in order to make weak seroreactions specifically stronger and thus the discover the nature of the reaction. Of these means and the results obtained when applying them Doepfmer (1951) gives a good illustration. On the basis of the literature he enumerates the following substances and measures which have been used to this effect: salvarsan and other related compounds, calomel, iodine, milk, bismuth, gold, malaria, "Organluin" (a preparation obtained from syphilitic rabbit testicle), palligen, emetic stone, thyroidin, "provocatin" (adrenalin + ephedrin + adenosin phosphoric acid), autotransfusion, typhoid vaccination, X-ray treatment, UKW, distilled water in the spinal canal and penicillin. To this list might be added intravenous vitamin K injections (Buccellato 1948, Corrente 1949), intravenously administered alcohol (Deme 1950) and urotropin (Resta 1950). As the positive results obtained by these different methods varied from 10 to 100 % and the optimum time at which the reaction result should be re-checked varied with the same substances with different investigators from hours to months, Doepfmer is of the opinion that a most variable phenomenon to say the least, must be in question.

Numerous investigators at present emphasize the value of quantitative serologic investigations in distinguishing between false and specific positive reactions. The general opinion is that in false positive sera the titer is low and fluctuating as well as apt to turn negative rather rapidly (*e.g.*, Stokes, Boerner, Hitchens and Nemser 1946, Reznikova and Ashavskaia 1949, Brede 1951 *a*, Rein 1951). Even high titer values are nevertheless found also in false positive sera (*e.g.*, Stokes and James 1949). Young (1943) is of the opinion that the strength in the Kahn reaction is connected with stability only when a "chronic" syphilis infection is in question.

In conclusion, it may be stated on the basis of the literature that false positive syphilis reactions are continuously obtained in many different diseases and conditions. The mechanism of their

origin has not yet been fully explained. It is certain that some of them are caused by technical errors and others by biological reasons. Evidently no homogeneous basis exists for these biological reasons. No fully satisfactory means have yet been devised for distinguishing false reactions from specific reactions.

On Quantitative Seroreactions for Syphilis

The recent literature unanimously supports quantitatively performed seroreactions for syphilis (*e.g.*, STOKES, BOERNER, HITCHENS and NEMSER 1946, MAILLARD and ORZEL 1946, THOMAS 1946, REZNIKOVA and ASHAVSKAIA 1949, KÁLDOR and SOMOS 1950, REIN 1951). REIN (1951) emphasizes the fact that a patient whose seroreaction titer is higher than that of another patient, is not necessarily more "syphilitic" than the latter. He lists the following situations in which quantitative investigations are of value: 1) in observing the response to treatment; 2) in distinguishing between serological relapse and re-infection; 3) in distinguishing between prenatal syphilis and syphilotoxaemia; 4) in verifying seroresistance, and 5) in distinguishing between specific and false positive reactions.

THOMSEN (1925) in investigating the behaviour of the quantitative Wassermann during treatment was of the opinion that in acquired syphilis the titer begins decreasing only after 2—3 weeks from the beginning of the treatment; if the reagin titer has not decreased at all, this in his opinion indicates congenital syphilis. THOMAS (1946) holds the opinion that if latent syphilis more than 6 months old is concerned, it takes at least one year before a full seronegativity is achieved. In general, the flocculation reactions become negative later than the complement fixations reactions (*e.g.*, OLIN 1935). THOMAS (1946) is also of the opinion that if the titer of the complement fixation reactions is > 20 units or the titer of the flocculation reactions > 32 units nine months after treatment, the treatment must be resumed; if the cerebrospinal fluid finding is negative but a reaction < 10 units persists in the blood for years no treatment is indicated merely because of this reaction. WALKER and STOUGHTON (1951) recommend that a complete penicillin treatment be given to all pregnant women

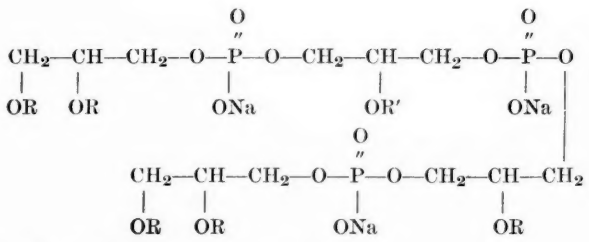
who have once been syphilitic and treated, if their Kahn titer is > 16 units. HUSSELS, F. (1949) also suspects serological relapse if one year after the completion of the treatment the Kahn titer rises to > 16 units. Regarding congenital syphilis WALKER and STOUGHTON (1951) start treatment if the seroreaction titers of the child of a clinically asymptomatic mother do not show a decreasing trend in three months from the delivery. The Wassermann titer of seroresistent cases is less often > 1 : 5 (KÁLDOR and SOMOS 1948).

To REIN's above mentioned list we might add the significance of the quantitative studies in the study of the "zone phenomenon". This phenomenon has been explained by DEAN and WEBB (1926). They observed that with certain antigen-antibody ratios the quickest and strongest reactions were obtained; they called this proportion the "optimum ratio". With a certain antigen this ratio remained unchanged in all dilutions. The phenomenon may lead to false negative results in the serologic syphilis diagnostics (*e.g.*, BEELAR, ZIMMERMAN and MANCHESTER 1949, SCHWANK 1950). In primary syphilis this phenomenon has not been encountered (*e.g.*, SCHWANK 1950). In RUGE's (1951) material of 2,585 quantitative Wassermanns this phenomenon appeared in 4 latent syphilis and tabes dorsalis cases, *i.e.* the routine Wassermann in the dilution 1 : 5 was negative, but because positive with increasing dilution of the serum, and in 17 cases the routine Wassermann was weakly positive, but in quantitative examination the titer obtained was nevertheless 1 : 128. In SCHWANK's (1950) material of 112 untreated syphilis cases the phenomenon could be established in 7 cases.

On the New Syphilis Antigens

On Cardiolipin and its Use as Syphilis Antigen

As mentioned before, in 1941 PANGBORN isolated from beef heart a new phospholipid to which she gave the name cardiolipin. It proved to be serologically inactive alone, but acquired antigen properties when used together with lecithin. On the basis of hydrolytic investigation PANGBORN (1947) obtained the following probable structure for cardiolipin:



R = Linoleylradical, R′ = Oleylradical.

Hence, cardiolipin is a phospholipid containing no nitrogen, but containing free acidic valences of phosphoric acid. Therefore it can form true salts in the form in which it is present in the tissues (PANGBORN 1951). The above therefore, illustrates the Na salt of cardiolipin. However, the structure formula is only hypothetical and PANGBORN (1951) emphasizes that there may appear variations which cannot be determined from the chemical analysis. For example, instead of the α-ester β-ester might enter into question, the isomeres of which, however, would probably be so closely related in their properties that their chemical differentiation would be impossible. The combination of the fatty acids might also de different. This is indicated by the iodine number, but the variations found in it are not very large, and it is difficult to say in each case whether the low iodine number might be due to oxidation during the preparation. The only fatty acids identifiable by hydrolysis are the linoleic and oleic acids. Experimental investigations have proved that cardiolipin can be divided into two different components, of which one contains a little less carbon that the other (*e.g.*, FAURE and COULON 1948, RICE and OSLER 1950). PANGBORN (1951) mentions that the many different cardiolipin lots prepared in their laboratories in different years have been serologically so constant that it is difficult to believe that these two cardiolipin components, divided up according to their different carbon content, are serologically different, and RICE and OSLER (1950) have also been able to verify that both of these components are serologically active. That the syphilis antigen properties of the beef-heart extract are not based only on the phosphoric compounds contained in it has been shown by UROMA, TOMMILA and LOUHIVUORI (1951) by isolating from the beef heart a phosphorus-free, but nitrogen-containing substance which when used together with cholesterol and lecithin has the proporties of a syph-

ilis antigen. A feature common to this substance isolated by them and to cardiolipin is that they are both unsaturated compounds and relatively slightly alcohol-soluble.

The preparation and purification methods for cardiolipin have been explained in detail in the monograph on the cardiolipin antigen published by WHO (PANGBORN, MALTANER, TOMPKINS, BEECHER, THOMPSON and FLYNN 1951) and therefore only the main elements of this complex process are given here.

Purified and ground beef heart muscle is twice extracted with acetone, the residue dissolved in methanol. From the methanol solution cardiolipin is precipitated with $BaCl_2$, in this phase lecithin remains in the solution. Barium precipitate, still containing many impurities, is suspended in ether and washed with acetic acid upon which the ether solution must be clear. Methanol and $BaCl_2$ mixture is added, centrifuged, the precipitate is dissolved in a small quantity of ether, which is then poured into methanol. The ether-methanol solution then contains ca. 50 % of the original crude precipitate. The precipitate contains barium cardiolipin and other strongly acidic phospholipids. In order to remove these the Ba salts are converted to Na salts and the methanol-soluble Na salts are again precipitated with $BaCl_2$. The precipitate is washed with methanol and acetone, ether is added and if no typical stiff gel is produced, more acetone is added, then centrifuged, and the precipitate is again dissolved in ether; this is repeated several times; the gel usually develops during the first precipitations. Acetone is then added until the gel becomes a solid precipitate. The precipitate obtained, which already contains almost pure cardiolipin in the form of Ba salt, is dissolved in ether-ethanol and converted to Na-salt. The Na-salt of cardiolipin is precipitated from the ethanol solution with $CdCl_2$ and the precipitate obtained is reprecipitated several times from the ether. Finally it is dissolved in a small quantity of ether, acetone is added, the solution is centrifuged; the precipitate obtained is dissolved in ether-ethanol and converted to Na-salt, which is dissolved in anhydrous ethanol, this is allowed to stand for a few days at 3 — 6° C, whereupon it is again centrifuged. The clear solution thus obtained is used in the preparation of the cardiolipin antigen. The solution keeps at room temperature provided it is protected from light. According to the above mentioned monograph the following requirements are made of the chemistry of this solution: the phosphorus content must be 4.15 % — 4.25 %, it may contain a maximum of 0.1 % nitrogen and the iodine number must be 115 — 127; in addition the Ba and Ca salts should form a typical gel in anhydrous ether.

Cardiolipin alone, as has been mentioned previously, is serologically inactive and acquires its antigen properties only when used together with lecithin. The preparation of lecithin, therefore, is another important problem in the preparation of the cardiolipin

antigen. The purification of lecithin presents certain difficulties. It is a neutral compound which forms only one satisfactorily known derivative — a double salt or complex with $CdCl_2$, the only form which can be utilized in purification; thus no true salt precipitations can enter into question. Moreover, lecithin tends to retain other compounds in solution (PANGBORN 1951). Lecithin appears in practically all types of plant and animal cells. Comparative investigations have shown that the beef heart muscle lecithin and egg lecithin are serologically of equal value (PANGBORN 1951, FLYNN, TOMPKINS and BEECHER 1952). Earlier serological investigations were made with the cardiolipin antigen containing beef heart muscle lecithin. PANGBORN (1951) however, is of the opinion that it would be simpler to go over entirely to the use of egg lecithin, since its purification is much easier in every respect.

The preparation of beef heart muscle lecithin, according to PANGBORN, is roughly as follows: 1. After cardiolipin has been precipitated with $BaCl_2$ from the methanol solution containing the methanol-soluble beef heart muscle components remaining from the acetone extraction lecithin is precipitated from the remaining methanol solution with $CdCl_2$, the Cd salt is then precipitated three times from $CHCl_3$ into C_2H_5OH. 2. Cd removed, free lecithin dissolved in ether. Ether solution chilled and precipitate discarded. 3. Lecithin in C_2H_5OH + $Ba(OH)_2$ and CO_2. Discard precipitate. 4. Solution from (3) reprecipitated with $CdCl_3$. 5. Cd precipitate from (4) twice precipitated from $CHCl_3$ into C_2H_5OH. 6. Cd precipitate from (5) treated once with petroleum ether and 80 % alcohol. Concentration of alcoholic layer → precipitate of Cd lecithin 7. Cd removed and cold-ether treatment repeated. 8. Purified free lecithin dissolved in absolute alcohol.

The preparation of egg lecithin again is according to PANGBORN (1951) in principle the following: 1. Cd salt four times precipitated from $CHCl_3$ into C_2H_5OH. 2. Precipitate from (1) treated once with petroleum ether and 80 % alcohol. 3. Cd removed, free lecithin dissolved in ether, ether solution chilled and precipitate discarded. 4. Purified free lecithin dissolved in absolute alcohol. The facility of the preparation as compared with the beef heart muscle lecithin is evident.

A third substance in the composition of cardiolipin antigen is cholesterol, available commercially in an adequately pure form; thus its acquisition presents no difficulties.

Cardiolipin lots prepared according to the same formula have hardly any serological differences (KAHN and MCDERMOTT 1948 *b*, PANGBORN 1951). The lecithin lots prepared at different times differ to such an extent, however, that according to KAHN

and McDERTMOTT (1948 *b*) each antigen which contains new lecithin must be standardized serologically. Several investigators hold the opinion that a special optimum lecithin concentration must be determined serologically for each antigen lot (*e.g.*, HARRIS, ROSENBERG and DEL VECCHIO 1948 *a*, KAHN and McDERMOTT 1948 *b*). PANGBORN (1951) is of the opinion that the antigen formula must always be kept the same and the antigen components must be purified until the standard reactivity is attained.

Diversity of opinion obtains on the different components of the antigen. MALTANER and MALTANER (1945) used in the complement fixation reaction an antigen in which lecithin/cardiolipin was 5/1 and cholesterol/lecithin 3.4/1. KLINE (1946) used in the slide test an antigen in which lecithin/cardiolipin was 9.4/1, REIN and BOSSAK (1946 *a*) again, in their slide test, arrived at the ratio 6.5/1. When using cardiolipin in his own reaction KAHN (1948) arrived at an antigen which contained 1 % of lecithin, 0.1 % of cardiolipin and 0.025 % of cholesterol. KLEIN and LEIBY (1948) used lecithin and cardiolipin in the ratio 8/1. KOLMER and LYNCH (1948) used in the Kolmer reaction an antigen in which the percentages for cardiolipin, lecithin and cholesterol were 0.03 — 0.05 — 0.6. GIORDANO, FROST and HIGGINBOTHAM (1949) used in the Kolmer reaction a cardiolipin antigen which contained 0.06 % of cardiolipin, 0.05 % of lecithin and 0.9 % of cholesterol.

The dilutions in which the different investigators have used the cardiolipin antigen in the complement fixation reactions also vary. KOLMER and LYNCH (1948) used a dilution 1 : 150, VOGELSANG (1948) a dilution 1 : 130. GIORDANO, FROST and HIGGINBOTHAM (1949) diluted their antigen in the ratio 1 : 650. BEKIERKUNST and MILGROM (1950) found the best dilution to be 1 : 67, and RUGE (1951), the dilution 1 : 133. The Pasteur Institute in Paris recommends its cardiolipin antigen to be used in the Wassermann reaction diluted 1 : 100.

HARRIS and PORTNOY (1944) used the cardiolipin antigen for the first time in the Kolmer complement fixation reaction. Important investigations into the use of the cardiolipin antigen in the complement fixation reaction have also been made by MALTANER and MALTANER (1945). In the macroflocculation reaction the cardiolipin antigen was used for the first time by BROWN (1944). The slide test with the cardiolipin antigen has been developed by KLINE

(1946) and by REIN and BOSSAK (1946 *a*) as well as by the investigators of the Venereal Disease Research Laboratory. Innummerable other slide test modifications have also been reported but it is hardly necessary to discuss than all.

Some Features of Serological Investigations Carried out with the Cardiolipin Antigen

The serological literature on cardiolipin has already become so extensive that a comprehensive survey of it would require too much space. A few examples only will be given here.

KLINE (1946) when examining 650 syphilitic sera, obtained the largest number of positive results with the cardiolipin antigen. In a total of 270 malaria cases, he had one positive result with cardiolipin, 24 with the Mazzini reaction and 8 with the Kahn reaction. Of 1,600 nonsyphilitic samples none reacted positively with cardiolipin, whereas 4 yielded a positive result with the Kline antigen. REIN and BOSSAK (1946 *a*) investigated with their own slide test method 600 samples from 60 syphilities. The cardiolipin antigen gave a positive result in 88 % of the cases, the Kline diagnostic reaction in 37 % and the Mazzini reaction in 51 %. In 37 cases of leprosy the cardiolipin antigen yielded a positive result in 51.5 %, Kahn in 62.0 % and Kolmer in 79.5 %. They also investigated, using cardiolipin, 39 cases of malaria which yielded positive results with some of the other antigens. Of these the sera of 29 cases reacted negatively with cardiolipin and the sera of 10 cases positively. Of these 10, 6 proved to have syphilis in the medical history. In syphiloid infections cardiolipin behaved in the same manner as in syphilitic sera. When investigating the inhibition reaction developed by them VOLKIN, NEURATH and CRAIG (1947) observed the phenomenon to be more specific when using the cardiolipin antigen than with Kahn's presumptive antigen. GIORDANO, CULBERTSON and HIGGINBOTHAM (1948) compared the Mazzini and the cardiolipin slide test applying the VDRL method. Of their material, comprising 24,085 sera, 97.07 % yielded a similar and 99.58 % a partly similar result for both reactions. In the group of the cases where the results were different, 8 were biologically false and 62 specifically positive results with the cardiolipin antigen; for the Mazzini the correspon-

ding figures were 13 and 7 respectively. HARRIS, ROSENBERG and DEL VECCHIO (1948 *b*) compared the cardiolipin antigen in the slide and tube tests. The results were similar in 98.5 %. They are of the opinion that the tube test cannot replace the slide test. KENT, CULWELL, COATNEY and COOPER (1948) studied several samples from 104 nonsyphilitic malaria patients; the Kahn reaction yielded 116 false positive results while the corresponding figure for the slide test was 14 with the Rein — Bossak technique, and 15 with the VDRL technique. KLEIN, LEIBY and BERKE (1948) compared the cardiolipin slide test (Kline's technique), the Kahn reaction and the Kolmer complement fixation reaction. In 205 syphilitic sera the cardiolipin antigen yielded a positive result in 98 %, Kahn in 83 % and Kolmer in 91.2 %. In 4,453 nonsyphilitic samples the cardiolipin antigen gave a false positive result in 0.29 % and the Kahn reaction in 0.11 %. LEVINE, KLINE and SUESSENGUTH (1948) obtained in 24,511 nonsyphilitic cases a false positive response with the cardiolipin antigen in 7 cases as compared with 67 false results in Kline's diagnostic and 231 in Kline's exclusion reaction. When employing cardiolipin as the antigen in the macroflocculation reaction in cerebrospinal fluid investigations, ROSENBERG, HARRIS and HARDING (1948) found it similar to the other antigens in sensitivity. VOGELSANG (1948) used cardiolipin as the antigen in the Wassermann reaction and compared it with the beef heart antigen usually employed by him in the Wassermann reaction, with the Kahn reaction and with Meinicke's second clarification reaction. His material covered 5,630 sera, of which 700 were derived from syphilities and 4,930 from nonsyphilities. Of these 700 syphilitic sera, 401 gave a positive result for the Wassermann reaction performed with cardiolipin and 367 when the standard Wassermann antigen was used. The Kahn reaction yielded 378 positive results and Meinicke's second clarification reaction 432. The cardiolipin Wassermann yielded 36 false positive results, the standard Wassermann 59, Kahn 51 and Meinicke's second clarification reaction 67. It should be mentioned that the beef heart antigen used by VOGELSANG in the Wassermann reaction contains 1 % of cholesterol, which KRAG and VOGELSANG (1948) consider to be the reason why the Wassermann results obtained in Copenhagen and Bergen were divergent; the Wassermann antigen used in Copenhagen contained 0.26 % of cholesterol. WIDELOCK (1948) compared the Mazzini, Kahn and

Kolmer reactions with the slide test (VDRL technique) performed with the cardiolipin antigen. Of 1,210 sera known to be syphilitic, 17 — 18 % yielded a negative Kahn and Kolmer where the Mazzini reaction and the cardiolipin slide test were positive. In the series of 8,519 Mazzini-positive sera he obtained positive reactions with Kahn in 57.4 %, with Kolmer in 64.2 % and with the cardiolipin slide test in 83.6 %. For the cardiolipin slide test performed according to the Kline method DEGÓES and MARTINO (1949) obtained positive results in 19.6 % of their investigation series as compared with 18.6 % for the Kahn reaction and 18 % for the complement fixation reaction. GIORDANO, FROST and HIGGINBOTHAM (1949) compared the Kolmer reactions performed with the cardiolipin and Kolmer antigens in a series of 1,028 sera. They obtained in 77.1 % completely similar, in 5.6 % partly similar, and in 17.3 % divergent results. The Kolmer antigen yielded no false positive results, with the cardiolipin antigen there were 0.9 % false positive results. They also compared the results yielded by the cardiolipin antigen in the Kolmer reaction and in the macroflocculation reaction, obtaining in 73.9 % completely similar, in 10.2 % partly similar, and in 15.9 % divergent results. LUNDBÄCK (1949) used the cardiolipin antigen in the Wassermann reaction and compared the titers obtained with it and with the Mørch extract. Of 297 syphilitic sera, 230 (78 %) gave a higher titer with cardiolipin. Of the 39 sera from syphilities in the primary stage 24 yielded a higher titer with the Mørch extract. MCDEARMAN and COTTRELL (1949) studied 15,019 sera using the Kahn and Kolmer reactions as well as the cardiolipin slide test and the complement fixation reaction with cardiolipin as the antigen. 991 sera from 654 persons yielded a positive result for one or the other of the cardiolipin techniques where the Kahn and Kolmer were negative. Of these 654 persons 196 had syphilis, 258 were nonsyphilities and 200 were undetermined. Of the sera reacting most weakly 26.5 % were derived from the syphilis group and 73.5 % from the nonsyphilitic group. One half of the weak positive reactions had been obtained with the slide test alone. PRICE (1949) compared the Wassermann reactions he had performed applying the Harrison — Wyler technique with the cardiolipin Wassermann in 1,982 sera obtaining a similar result in 96 %. Of the 80 sera for which different results were obtained 4 were derived from primary syph-

ilities and 65 from treated syphilities, in all of which the cardiolipin Wassermann was positive, but the standard Harrison — Wyler Wassermann negative; 11 were apparently false positive reactions. On the basis of his investigation PRICE comes to the conclusion that the cardiolipin Wassermann is more sensitive than, but equally specific as the standard Wassermann. Incidentally, of his sera derived from 31 primary syphilities 18 gave a positive result with the cardiolipin Wassermann whereas the standard Wassermann was positive only in 14 cases, which was an opposite result to LUNDBÄCK's (1949) observation which has previously been mentioned. PRICE also made quantitative researches and observed that the cardiolipin Wassermann gave positive results in higher serum dilutions than the standard Wassermann. BEKIERKUNST and MILGROM (1950) compared the cardiolipin Wassermann with the Kahn reaction. Of 260 syphilitic sera the cardiolipin Wassermann yielded a positive result in 88.1 % and the Kahn in 86.2 %. Without clinically observable syphilis manifestations the cardiolipin Wassermann was positive in 13 and the Kahn in 14 cases, the percentages being 0.57 and 0.62 respectively. JAYEWARDENE and VELAUDAPILLAI (1950) compared the cardiolipin slide test with the Kahn and Wassermann reactions. The Wassermann yielded positive results in 44.4 %, the Kahn in 52.1 % and the cardiolipin slide test in 64.8 %. In 1,104 control sera the Wassermann reacted positively in 14.4 %, the Kahn in 10.4 % and the cardiolipin slide test in 14.5 %. These high percentages of false reactions are due, according to them, to the quality of the control material. They also state that the cardiolipin antigen was "the most sensitive in detecting proved early cases of syphilis". With their cardiolipin slide test KLEIN, KONWALER, SEARS, BERKE and LEIBY (1950), in 658 syphilitic sera, obtained positive results in 98.1 % as compared with 80.6 % for the Kahn reaction. In 13,304 nonsyphilitic sera the cardiolipin slide test yielded a specificity of 98.9 % as compared with 99.3 % of the Kahn reaction. PONTECORVO (1950) on the basis of his investigations arrived at the conclusion that the cardiolipin antigen is better in the Wassermann reaction than the standard beef heart antigen; as the antigen in the Kahn reaction it also gave good results, although it was somewhat inferior to Meinicke's clarification reaction. SPIELMANN's (1950) material comprised 446 syphilitic and 2,500 nonsyphilitic sera on which he

made both the cardiolipin slide test and the cardiolipin complement fixation reaction (Kolmer's technique), the standard Wassermann reaction, the Kahn reaction, Müller's turbidity reaction and the citochol reaction. In syphilitic sera the two cardiolipin reactions were positive in 88 % and negative in 1 % only. The Wassermann was negative in 35.2 %, the Kahn in 23.7 %, the Müller turbidity reaction similarly in 23.7 % and the citochol in 7.4 %. Of the 2,500 nonsyphilitic sera 133 yielded a false positive result for one or more reactions. In these the cardiolipin reactions were negative in 64.7 %, the Wassermann gave a positive reaction in 67 cases and the Kahn in 113. RUGE (1951) compared the results obtained for the Wassermann reaction performed with the cardiolipin antigen, the antigen prepared from syphilitic liver and the beef heart muscle antigen. There were in all 1,814 positively reacting sera. Of these the cardiolipin antigen gave a positive result in 97.9 %, the antigen prepared from syphilitic liver in 65.8 % and the beef heart muscle antigen in 58.8 %. With the cardiolipin antigen 6 strong and 12 weak false positive reactions were obtained, a total of 18, *i.e.* 0.3 %. With the other antigens 11 false positive reactions were obtained (0.2 %). In the same serum series Meinicke's second clarification reaction yielded false positive results in 0.5 %. The percentage of the false positive results given by Meinicke's reaction was 71.9 as compared with 74.7 for the cardiolipin Wassermann reaction.

Favourable results with the cardiolipin antigen are further reported by several other investigators, *e.g.*, DAMMIN and SCOTT (1948), LEVIN (1948), DORSEY (1949), HINTON, STUART and GRANT (1949), HASSELMANN (1950 *a*, *b*), ORPWOOD and WILKINSON (1950), BREDE (1951 *a*, *b*).

The investigators are not unanimous as to the superiority of the cardiolipin antigen to the other antigens. At least in the literature studies can be seen in which the results obtained with certain other antigens have been fully comparable with the results obtained with the cardiolipin antigen. KAHN and MCDERMOTT (1948 *a*) compared the results obtained with the Kahn standard antigen, the cardiolipin antigen in the slide test and the complement fixation reaction. In syphilitic sera the sensitivity of the Kahn reaction was 81.6 %, that of the cardiolipin slide test 75.0 % and that of the complement fixation reaction 73.3 %. The specificity in their material was

100 % for all the reactions. When evaluating the preliminary results received with the new antigens ZWALLY (1948) stated his opinion that in routine use they will hardly remove the hazards which always occur in serological investigations. HUSSELS, H. (1949) compared the cardiolipin and the Kahn standard antigens he had received from the Venereal Diseases Research Laboratory. The cardiolipin antigen he used in the slide test (VDRL technique). In fresh syphilis the sensitivity of the Kahn reaction was 98.5 % and that of the cardiolipin slide test 90.7 %, in old syphilis the corresponding percentages were 44.9 and 40.7 respectively. The specificity of the Kahn reaction was 96.8 % as compared with 84.5 % of the cardiolipin slide test. He is of the opinion that syphilis cannot be diagnosed if the cardiolipin antigen alone gives a positive result. SILLIPHANT, ENGELFRIED, ELLIS and BUTTERFIELD (1949) also compared two different cardiolipin slide tests and the Kahn reaction. The Kahn reaction was much more sensitive than the slide tests, although it also yielded more false positive results. BOAS (1950 *a*, *b*) investigated 522 syphilitic and 152 control sera using the cardiolipin antigen in the Kahn reaction as well as the standard Kahn antigen and the Kahn antigen manufactured by the Schering factories. The control sera were negative with all the antigens. The Kahn standard antigen gave positive results for syphilitic sera in 210 cases, the cardiolipin antigen in 206 cases and the Schering Kahn antigen in 198 cases. The sera in which divergent results were obtained were derived from latent syphilis patients. BOAS also made quantitative investigations which yielded similar results to the qualitative investigations, in other words, the Kahn standard antigen gave positive results in higher dilutions than the cardiolipin antigen and the titers given by this were again on the average higher than those received with the Schering Kahn antigen. With healthy animals the cardiolipin antigen gives as many positive results as the Kahn and Wassermann reactions (DEGÓES 1950). ZIA, CHAO and CHIANG (1951) investigated 1,163 sera in which the Kahn reaction yielded positive results in 335 cases, the VDRL cardiolipin slide test in 310 cases and the Kolmer reaction in 306 cases. When investigating the incidence of false positive reactions among school pupils STOUT, GUZMÁN and SCRIMSHAW (1952) employed the Kahn, Mazzini and Kolmer reactions as well as two cardiolipin slide tests (VDRL and Kline). The Kahn was positive in 9.1 %, the Mazzini

in 14.0 % the Kolmer in 2.4 % and the two cardiolipin slide tests in 1.7 %. As 6 % of all reactions were presumptive false positive and 5 % possible syphilitic the Kahn reaction, for example, yielded more positive results as compared with the cardiolipin test, but it also yielded more false positive reactions.

To sum up it may be said that cardiolipin as such is a serologically stable substance, the chemistry and the antigen properties of which have not, evidently, been finally clarified. The difficulties in the preparation of the cardiolipin antigen are at present primarily connected with lecithin. In the antigen formulas of the cardiolipin antigens considerable diversity exists. In a large proportion of the comparative serologic investigations into cardiolipin antigen, the cardiolipin antigen has proved to be more sensitive than the other antigens used in the investigations without specificity being reduced. Regarding the flocculation reactions there exist a great number of investigations in which the cardiolipin antigen is in no way, or only slightly more reliable than the other antigens used.

On Sitolipin and Sitolipin Antigen

Sitolipin is a phospholipid isolated from wheat germs, the chemical structure of which has not been fully explained as yet. It contains over 50 % of unsaturated fatty acids and 4.26 % of phosphorus, the iodine number being 119.5; it contains no nitrogen. Thus in its structure sitolipin is evidently very similar to cardiolipin (Uroma and Louhivuori 1951). Sitolipin alone is serologically inactive. The sitolipin antigen is composed of sitolipin, lecithin and cholesterol (Uroma and Tuomioja 1950).

The preparation of sitolipin according to Uroma and Louhivuori (1951) is, in the main, as follows: Wheat germ powder not more than three days old is used as a basis. This is extracted twice with acetone. The germ powder paste obtained is extracted a number of times with methanol and the methanol lots are combined. From the methanol solution sitolipin is precipitated with $BaCl_2$, the precipitate is washed with acetone and methanol, suspended in ether and converted by adding Na_2SO_4 solution to Na salt. The ether solution, which contains sitolipin as Na salt is concentrated and combined with 8 times the quantity of methanol. From the methanol solution obtained, sitolipin is again precipitated with $BaCl_2$ and the precipitate obtained is dissolved in ether, from which it is several times precipitated with acetone. The last precipitate obtained with acetone is dissolved in ether containing 10 % ethanol and is again con-

verted into Na salt by adding half saturated NaCl sitolipin. The ether solution which contains sitolipin as Na salt is dried with Na_2SO_4 and evaporated in vacuo and CO_2 current. The dry paste thus obtained is dissolved immediately at 57° C in absolute ethanol. The solution is allowed to stand for a few days at a temperature of 2—4° C and the slight precipitate formed during this time is removed by centrifugation. In this form sitolipin is already utilizable for antigen preparation. The alcohol solution contains ca. 3 mg/ml of Na sitolipin. At this stage sitolipin contains ca. 0.05 % nitrogen and some 4 % phosphorus. In order to purify the sitolipin further it is precipitated from this ethanol solution with $CdCl_2$, the precipitate obtained is dissolved in ether, from which it is twice precipitated with acetone. Thereupon the sitolipin is once more converted into Na salt, which is dissolved in absolute ethanol under simultaneous heating to 57° C. Only a small powderlike residue remains undissolved. The solution is again allowed to stand for a few days at 2—4° C and the colourless solution is isolated by decantation from any small precipitate. The solution obtained is ready to be used as antigen. It no longer contains nitrogen. The solubility of this Na sitolipin in absolute ethanol is about 3 mg/ml, it contains 4.0 % phosphorus, and its iodine number is 115. The antigen properties of the solution remain unchanged at room temperature, when protected from light, for over a year. Stored dry, sitolipin rapidly loses its serologic properties. The quantity of sitolipin obtained from one kilogram of wheat embryo flour provides sufficient antigen for 250,000—300,000 tube tests (Uroma 1951).

In their preliminary report Uroma and Tuomioja (1950) stated that they had used an antigen which contained sitolipin, lecithin and cholesterol in the ratio of 3 : 40 : 90. Later they used an antigen containing 0.05 % sitolipin, 0.3 % lecithin and 0.9 % cholesterol. After obtaining sitolipin in pure form they finally (1951 *a*) arrived at an antigen containing 0.02 % sitolipin, 0.15 % lecithin and 0.9 % cholesterol.

Uroma and Tuomioja (1950) used the sitolipin antigen in the VDRL tube test. Tuomioja (1951) also used it in the Wassermann reaction diluted in the same manner as in the flocculation reaction. Rein, Kelcec and Rosenfield (1951) used the sitolipin antigen both in the slide test and in the complement fixation reaction.

Some Features of Serologic Investigations Carried out with Sitolipin Antigen

Uroma and Tuomioja (1950) investigated 390 sera, comparing the VDRL tube test with sitolipin and cardiolipin antigens with the Kahn reaction. 107 reacted positively for one or more reaction.

Of these the sitolipin antigen yielded positive results in 99 cases, the cardiolipin antigen in 95 and the Kahn reaction in 84 cases. UROMA (1951) compared the results obtained for the Wassermann reaction, the Wassermann reaction sensitized with cholesterol, the Kahn reaction and the VDRL tube test with the sitolipin antigen. The material comprised 2,841 sera, of which 2,553 were negative for all reactions. Of the 288 positive sera the Wassermann gave positive results in 56 cases, the Wassermann sensitized with cholesterol in 176, the Kahn in 268 and the sitolipin test in 283 cases. 20 sera yielded positive results for the sitolipin test alone and 5 sera for the Kahn reaction alone. All other reactions were specific except for two Kahn-positive sera which derived from patients in whom syphilis could not be diagnosed. TUOMIOJA (1951) examined 1,318 sera using sitolipin as the antigen in the Wassermann reaction and compared the results he obtained with the results obtained when using the standard Wassermann antigen, the Wassermann antigen sensitized with cholesterol, and the Kahn reaction. A total of 192 sera proved to be Kahn-positive. Of these, the standard Wassermann antigen yielded a positive result in 62 cases, the Wassermann antigen with cholesterol in 89 and the sitolipin antigen in 119 cases. False positive results were not separated from the true positive results. On the basis of a material of 10,000 sera UROMA and TUOMIOJA (1951 *a*) compared the results obtained for the Kahn reaction and the VDRL sitolipin tube test. Of these sera 9,818 reacted negatively in both reactions, while 146 yielded positive results for both. In addition, 29 sera gave positive results only in the sitolipin test, and 7, only for the Kahn reaction. Of the sera reacting positively only in the sitolipin test, 22 proved to be definite syphilitic sera, the remaining 7 sera derived from persons in whom syphilis could not be diagnosed. Of the sera that were only Kahn-positive, 6 were false positive and only one specific. UROMA and TUOMIOJA (1951 *c*) also made comparative quantitative investigations using the Kahn reaction and the sitolipin tube test. In 86 % of the investigated positive sera the sitolipin test yielded higher titer values than the Kahn reaction. The authors also compared the titer values obtained in the tube test with the sitolipin and cardiolipin antigens. In 89 % of the investigated sera the titer values were the same. On the basis of a material of 9,830 sera UROMA and TOMMILA (1951) compared the results obtained for

the sitolipin slide test and the Kahn reaction. These both gave a positive result in 132 sera, the Kahn alone was positive in 19 and the sitolipin test alone in 32 cases. Of the 19 cases in which the Kahn alone was positive, 18 had nothing indicative of syphilis. Of the 32 in which the sitolipin test alone was positive, 28 proved to be syphilitic. UROMA and SAVOLAINEN (1951) studied 1,448 blood samples from the patients in the Municipal Hospital for Contagious Diseases in Helsinki, and performed the Wassermann, cholesterol-Wassermann and Kahn reactions and the sitolipin tube test. The specimens investigated were derived from patients with various contagious diseases, the majority being samples from scarlatina patients (1,173). A total of 30 positively reacting cases were found, of which 6 had syphilis. The sitolipin tube test was positive in all the syphilis cases and yielded a false positive result in one case. The Kahn was negative in one case of syphilis, and gave a false positive result in 23 cases. The Wassermann was positive in 3 syphilis cases and false positive in 2 cases. The cholesterol-Wassermann gave positive results in 3 syphilis cases, false positive results in 13 cases. UROMA and TUOMIOJA (1951 *b*) also compared the results obtained for the Wassermann reaction and the sitolipin tube test in cerebrospinal fluid investigations. In addition to specimens which gave similar results, the sitolipin tube test alone was positive in 15 cases (all specific results) and the Wassermann alone in 3 cases. VOGELSANG (1952) investigated 793 syphilitic sera using his own beef heart antigen, containing 1 % of cholesterol, employed in Bergen as well as the cardiolipin and sitolipin antigens in the Wassermann reaction. In 762 sera all three antigens gave similar results. In the 31 sera in which the results differed, the beef heart antigen gave a positive result in 13 cases, the cardiolipin antigen in 21 cases and the sitolipin antigen in 10 cases. In the control material comprising 9,207 nonsyphilitic sera, the beef heart antigen gave 32 false positive results, the cardiolipin antigen 15 and the sitolipin antigen 12. REIN, KELCEC and ROSENFIELD (1951) compared the sitolipin and cardiolipin antigens in the VDRL and Rein — Bossak slide tests and the Kent complement fixation reaction. The VDRL slide test material was 1,682 sera, of which 371 reacted positively. A similar result with both antigens was obtained in 98 %, a partly similar result in 1.6 % and differing results in 0.4 %. The sitolipin antigen was 13 % more sensitive than the cardiolipin antigen. The Rein —

Bossak slide test material consisted of 1,798 sera of which 338 reacted positively. A completely similar result was obtained in 98.4 %, a partly similar result in 1.5 % and differing results in 0.1 %. The sitolipin antigen in this series was 3 % more sensitive than the cardiolipin antigen. The Kent complement fixation was carried out with both these antigens on 614 sera, of which 217 reacted positively. A completely similar result was obtained in 98.0 %, a partly similar result in 1.4 % and differing results in 0.6 %. This time the cardiolipin antigen was 3 % more sensitive than the sitolipin antigen. False positive results were not separated in any of the series.

No other serologic studies concerning the sitolipin antigen were available to me at the time of writing this study.

Summary: Sitolipin is a nitrogen-free phospholipid isolated from wheat germs, the chemical composition of which still awaits final solution. In structure sitolipin is evidently related to cardiolipin. Sitolipin alone is serologically inactive. In addition to sitolipin the sitolipin antigen contains lecithin and cholesterol. Serologic experiments made with this antigen seem to indicate that in both sensitivity and specificity the sitolipin antigen is entirely comparable with the cardiolipin antigen.

Other Syphilis Antigens of Vegetable Origin

Sitolipin is a proof that vegetable substances can be found which are identical with or at least closely related to certain substances employed in human serology. This trend in research has been followed for some years in the Department of Serology and Bacteriology of the University of Helsinki, where RENKONEN (1948) found in the seeds of some leguminous plants, for example, substances similar to the haemagglutinins in man.

The idea of isolating syphilis antigens from vegetable sources is not new. HEINLEIN (1912) described the syphilis antigen he had prepared from potatoes. TRIBONDEAU (1913) and WALKER (1917) reported good results with the syphilis antigen they had prepared from peas. WEISS (1923) studied a number of plants and seeds and observed that olive and coconut extracts contain a relatively large quantity of "syphilis antigen". The fact that soya beans contain a compar-

atively large amount of phosphatids (MARKLEY and GOSS 1944) induced STEVENSON (1949) to experiment with soya bean extracts as the syphilis antigen. When the soya oil had been pressed out of the soya beans an extraction was made of the remaining powder according to the Kahn standard method, and the extract so obtained was serologically active in syphilis. It became serologically active 18 — 36 hours after the preparation and rapidly became too sensitive and thus unfit for use after that period. As the extract prepared from whole soya beans was serologically inactive, STEVENSON thought it likely that soya oil may contain a substance which acts as inhibitor. STEVENSON (1950) used this antigen in the Kahn reaction and with it obtained similar results to those given by the standard Kahn reaction and the Wassermann reaction in 80 % of his specimens. The reactions were generally weaker than those obtained with the Kahn antigen. In four weeks the antigen became unfit for use. STEVENSON (1950) assumes that the soya extract is more heterogeneous than the beef heart antigen. He divides the impurities of the extract into two groups: a) those causing the high NaCl titer of the antigen (1 : 2.2), b) those which during storage make the antigen hypersensitive without altering the NaCl titer. By an improved extraction technique MACNAB (1950) was able to isolate from soya beans a fraction, which, together with albumin, acted as a syphilis antigen. He assumes that soya beans contain a substance which in its serologic activity is similar to cardiolipin. More extensive experiments regarding the value of these soya bean antigens in serologic syphilis diagnostics are not yet available in the literature.

The Berger Reaction

The Wassermann and Kahn reactions and Meinicke's second clarification reaction are so well known that the present author does not find it necessary to deal with the extensive literature on them. It may, however, be relevant to discuss the BERGER reaction, because it has been included in the present investigations and because it is less well known.

BERGER (1943) investigated the effect of 78 different stains added to the Kahn antigen. The following stains had a marked

sensitization effect: Janus green B, Victoria blue 4 R, Aniline blue, Isamine blue, Janus black, Methyl green, Nile blue sulphate, Pyronine G and Sudan black. The effect was best with the two first mentioned. The effect of the addition of stain is different from that of cholesterol. The sensitizing effect of cholesterol begins only some time after it has been added, while the sensitizing effect of stain appears immediately after it has been added. There is hardly any question of an electrolytic effect in the stain addition, as even with the electronegative isamine blue a sensitizing effect is produced. BERGER assumes that the sensitizing effect of stain is based on an occurrence resembling the antigen-antibody reaction.

BERGER describes in this same paper a slide test in which the Kahn standard antigen sensitized with Victoria blue is used as the antigen. The preparation of the antigen is as follows: To 10 parts of antigen 1 part of the British Pharmacopoeia tinct.benz.comp. is added. To 10 ml of this mixture 17 mg of Victoria blue is added. Out of this basic solution which keeps for years antigen emulsion is prepared by adding rapidly to 0.1 ml of the basic solution 1 ml of 0.85 % saline solution. This emulsion keeps for ca. 12 hours. To a drop of inactivated serum on the slide a drop of antigen emulsion of equal size is added, the glass is rotated for 4 minutes after which the reading of the reaction is taken macroscopically. FODDEN and MADDOCK (1947) used only 2 minutes rotation, since, according to them, a longer rotation might lead to misleading granularity.

In the same paper BERGER reports his investigation with this reaction of 1,112 sera, of which by means of the Wassermann (Wyler's modification) and Kahn reactions as well as the slide test described a total of 187 more or less clearly positive sera were detected. Berger's own reaction was clearly positive in 160 cases and weakly positive in 18 cases. With the Wassermann reaction the corresponding figures were 131 and 20, and with the Kahn reaction 124 and 19 respectively. In 26 cases the Berger reaction was positive when both Kahn and Wassermann were negative. Possible false positive reactions had not been specified. DIMLER (1949), in the Berger reaction, applied citochol extract instead of the Kahn standard extract. Among the 1,900 sera investigated by him there were, in addition to those yielding similar results for the Kahn and Berger reactions, 38 sera in which the Berger was positive but the Kahn negative. In 7 sera the reverse result was obtained. In the treated syphilis cases in his material the Berger

reaction remained positive for a longer time than the other flocculation reactions employed. MAGNUSSON (1950) investigated the relationship of the results obtained for the Berger reaction to the results obtained for the Wassermann and Kahn reactions and Meinicke's second clarification reaction. In syphilitic sera the Berger reaction was positive in 85 %, while the corresponding percentages were 60.5 for the Wassermann, 67.5 for the Kahn and 83.0 for Meinicke's second clarification reaction. In 1,796 non-syphilitic sera the Berger reaction yielded false positive results in 0.39 %, the Wassermann reaction in 0.17 %, the Kahn in 0.36 % and Meinicke's second clarification reaction in 0.89 %.

The Berger reaction, then, is a slide test in which an attempt has been made to increase the sensitivity of the Kahn extract used by the addition of Victoria blue. The few investigations into this reactions seem to indicate that it is more sensitive than the Kahn reaction. As far as is known, the part played by false positive results has been described in one study only, in which the number of false positive results in the Berger reaction roughly equalled that for the Kahn reaction.

THE PRESENT SEROLOGIC STUDIES

The Reactions Used and Their Techniques

The Standard Wassermann Reaction (WR) *:*

The technique employed was the same as has been used and described e.g. by SIEVERS 1937, ANTTONEN 1945 and PENTTINEN 1946. Hence, the main outlines only will be given in the following: *Preparation of the antigen:* To purified and ground beef heart 96 % alcohol was added in the ratio 1 : 10. The mixture was shaken for half an hour after which it was placed for 2 hours in a waterbath at 56° C. The mixture was allowed to cool and then filtered through filtration paper. The solution was then ready for use. If the previous lot of antigen had been exhausted, the new lot could be utilized immediately without any difference being noticed in its antigen properties. Immediately before the use of the extract as an antigen it was diluted with saline solution (0.9 %) in the proportion one part extract to five parts saline solution.

Complement: Guinea pig complement taken by cardiac puncture was used. No special "complement guinea pigs" were used. Liberal amounts of

* The abbreviation which will be used for the reaction is given in brackets.

blood were usually taken from the guinea pigs at one time and the complement was stored frozen. It was used in a 1: 10 dilution.

Amboceptor: Amboceptor titration was performed daily. In the reaction two and half times the amboceptor quantity needed for total hemolysis was used.

Cells: Red cells collected from sheep by jugular vein puncture and washed several times were used as a 5 % saline emulsion.

Sera to be tested: On the day of their arrival in the laboratory the sera, isolated from the whole blood by centrifuging, were inactivitated for half an hour at 56° C in a waterbath and then kept overnight in a refrigerator. In the morning a post-inactivation was carried out for 10 minutes before the test.

Reaction technique: In the test 0.1 cc of serum and all necessary reagents were used, the total volume thus being 0.5 cc. Each serum was added with its own pipette. The time of complement fixation was 75 minutes i.e. 45 minutes at room temperature and 30 minutes in a waterbath at 37° C. After the addition of blood cells and amboceptor the tubes were again allowed to stand at 37° C for about 20 minutes before the first reading of reactions was taken. Then the tubes were put back in the waterbath and after 40 minutes the second, last reaction reading was done.

The following controls were used: 1. Serum control where physiological saline replaced the antigen. 2. Positive control with a serum known to be positive. 3. Negative control with a serum known to be negative. 4. Antigen control containing physiological saline instead of serum, and 5. "Half control", similar to the last mentioned except that the complement quantity was reduced by one half. If the hemolysis in this tube was slow, it was considered to be a sign of sensitivity of the hemolytic system and slight inhibitions of the hemolysis in the test tubes were not considered. If the hemolysis in this tube was complete by the first reading, even a slight inhibition of the hemolysis in the test tubes was taken into consideration.

Reading of the reaction: The stage of the hemolysis was observed macroscopically. When the stage of the hemolysis was 0 — 40 % at the first reading and did not exceed 40 % by the second reading the result was marked +. If the stage of hemolysis at the first reading was 0 — 40 % and at the second reading 60 — 80 %, the result was given as ±. If the hemolysis was 80 — 100 % at the second reading the result was considered to be negative. Where necessary, such results could be indicated —? However, an attempt was made to avoid this way of indicating the reply, if possible. If the stage of hemolysis was already 60 — 100 % at the first reading, the reaction result was given as —.

The Wassermann Reaction Sensitized with Cholesterol (Chol. WR):

To the WR antigen 0.15 % of commercial powdered cholesterol was added. Otherwise the reaction was completely similar to the WR with regard to its reagents and technique as well as the interpretation of the results.

The Wassermann Reaction with Cardiolipin as the Antigen (Card. WR):

The cardiolipin antigen for the complement fixation reaction, prepared and kindly donated to us by the Pasteur Institute in Paris was used diluted with physiological saline in a ratio of 1 : 100. Otherwise the technique was exactly the same as for the WR.

The Wassermann Reaction with Sitolipin as the Antigen (Sitol. WR):

The sitolipin antigen prepared by the State Serum Institute and containing 0.9 % cholesterol, 0.3 % lecithin and 0.05 % sitolipin was used. All the antigen required was donated by the State Serum Institute. Sitolipin was also used diluted with physiological saline in the ratio of 1 : 100, a dilution which proved very satisfactory. The technique was otherwise like the WR.

The Quantitative Wassermann reactions with different antigens were all performed in the same manner. The sera were diluted with physiological saline so that the final dilutions were 1 : 10, 1 : 20, 1 : 40, etc. In other respects the technique was as described above.

In the Cerebrospinal Fluid examinations the same Wassermann technique was employed as in the serum investigations, the only difference being that each specimen was examined using two tubes. In one of these the amount of cerebrospinal fluid was 0.1 cc and in the other 0.2 cc.

The Kahn Reaction (Kahn):

Preparation of the Antigen: Beef heart with the fat removed was finely ground by passing it three times through a meat grinder. The paste was spread on a glass plate and dried in heat for 6 hours. It was then crushed, covered with gauze and allowed to dry until it could be ground in a mortar. 100 grams of this powder was put in a 1 liter Erlenmeyer flask, and 400 cc of anesthetic ether was added. It was shaken at short intervals during 10 minutes, whereupon the ether was removed by filtration. This procedure was repeated using 300 cc of ether for another three times. After the last filtration the mass was spread with a spatula on a piece of paper and dried to free it from ether odor. After this the mass was utilizable for alcohol extraction. The dry ether-less heart mass was weighed and placed in the original Erlenmeyer flask. 5 cc of minimum 95 % alcohol was added for every gram of powder. The flask was shaken for 10 minutes and then allowed to stand for three days at room temperature in the dark and without shaking. Immediately before the filtration it was shaken for 10 minutes. The filtrate was stored in the dark at room temperature. For the addition of cholesterol 6 mg of cholesterol, as pure as possible, were weighed for every cc of extract, this was then put in the flask and the alcohol extract added to it. The flask was placed in a waterbath and

rotated there until the cholesterol had dissolved. The solution was again filtered. Before application as the antigen it was titrated with various saline solutions in order to find out which of them gave the required opalescent dispersion. This dilution was then used in the reaction. The titrations performed with both positive and negative sera were compared with an antigen previously known.

Reaction technique: The extract was combined as rapidly as possible with physiological saline in the ratio observed to be best in the titration; generally this was 1 : 1 — 1.4. The antigen emulsion thus obtained was allowed to stand for 10 minutes, whereupon it was ready for use. 0.0125 and 0.025 cc of this antigen emulsion were pipetted with the Kahn pipette into two tubes for each serum to be tested. Then into both tubes was pipetted 0.15 cc of the serum which had been inactivated in the same manner as the sera to be used in the WR. There was a separate pipette for each serum. After the addition of serum there followed a short, vigorous shaking by hand, then the tubes were placed in a waterbath at 37° C for 10 minutes, and from there transferred to a shaking machine (250 oscillations per minute) for 3 minutes. After removal from the shaking machine 1.0 cc of physiological saline was added to the tubes with an automatic pipette. The reaction was then ready for reading.

Reading of the reaction: To read the reaction a magnifying loupe was used. For reporting the degrees of flocculation the symbols + + +, + +, +, —? were used. + indicates a fine flocculation only just visible to the naked eye, —? indicates a flocculation clearly visible only with the loupe. As controls known positive and negative sera are used as well as the saline control, in which serum is replaced by physiological saline. The results + + + and + + indicate clearly visible large and medium sized floccules in an otherwise clear medium. Where, in both tubes or even in one tube there was + + + or + + flocculation, the reaction was reported as +; where a flocculation was graded + in both or only one of the tubes (the other tube in the latter case is then —? or —) the reaction was reported as ±. Where the serum yielded a flocculation graded —? at the most, the result was given as negative. The final grading of the results is, thus: +, ± and —.

The VDRL Cardiolipin Slide Test (VDRL):

Antigen: The antigen was donated by the Venereal Disease Research Laboratory, as was the necessary buffered solution. The antigen contained 0.03 % cardiolipin, 0.9 % cholesterol and the amount of lecithin necessary for standard reactivity. The buffered solution consists of 1 % NaCl solution which has been buffered to pH 6.0 by means of formaldehyde and primary and secondary phosphate. The antigen emulsion needed was prepared separately for each day in the following manner: 0.4 cc buffered 1 % NaCl solution was pipetted into a round bottomed flask, 0.5 cc antigen was added by drops directly

onto the buffer solution while the flask was simultaneously slightly rotated on the plane; the dropping was effected in ca. 6 seconds. The pipette was blown empty and the rotation continued for 10 seconds longer. 4.1 cc of the buffer solution was added, and a glass or screw stopper put on the blotte. It was shaken vigorously for 10 seconds. The antigen emulsion was allowed to stand for about 1 — 2 hours before application (LUNDBÄCK and ALLANDER showed in 1951 that the cardiolipin-lecithin-cholesterol antigen must stand for a certain time after it has been brought to emulsion form. This time depends on the quality of the lecithin used, being 5 — 30 minutes with egg lecithin and 30 minutes with beef heart lecithin. The authors think that while standing the physico-chemical conditions may alter in such a way as to cause change in the character of the reacting surface of the antigen particles).

Reaction technique: Antigen emulsion was dispensed from a 2 cc syringe and a hypodermic needle gauge 1 held in a slanting position, so that a drop of constant size (1/60 cc) was obtained. The reactions were performed on 6×10 cm. glass slides on which, by means of a metal mould paraffin rings with an inside diameter of 1 cm had been made. 18 such rings may be made on one slide. After use the slides were washed with soap and warm water and the soap rinsed away with hot, not boiling, water.

As no shaking machine was available, it was necessary to resort to manual rotation. For this purpose a 50×50 cm plywood plate was prepared, and at the corners connected with another plywood plate of the same size by means of four 7 cm. long, stiff screw springs. The glass plates were arranged on the upper plate, for which a smooth rotating movement could easily be produced by hand. This movement roughly circumscribed a 4 cm. — radius circle 120 times per minute.

The reading of the tests was done with a loupe with $6 \times$ magnification; in the corroboration of weak reactions a microscope with $60 \times$ magnification was used.

0.05 cc of serum inactivated as for the WR was dropped into one paraffin ring. For each serum a separate pipette was used. With the syringe and hypodermic needle 1/60 cc of the antigen emulsion was added onto each serum. The slides were then rotated for 4 minutes, after which the reading of the tests was done immediately.

Reading of the reaction: The symbols used to indicate the degree of flocculation were: + + +, + + and +. + + + indicated definite clumps, clearly visible to the eye in a clear medium; + + indicated smaller clumps, just visible to the eye; + indicated small clumps visible only microscopically in an somewhat cloudy medium. In negative sera the antigen particles were seen as regular, rod-like formations of equal size. Known positive and negative sera were used by us as controls in the interpretation of the results. The final grading was reported as: +, ±, and —.

The degrees of flocculation + + + and + + were reported as +, the degree of flocculation +, as ± and others as —. The final grading thus corresponded with that used for the Kahn.

The Rein — Bossak Cardiolipin Slide Test (Rein — Bossak):

Antigen: The antigen was received from Dr. C. R. Rein in September, 1950. It contained 0.2 % cardiolipin and 1.3 % purified lecithin in absolute alcohol. The 1 % cholesterol solution needed was also received from Dr. Rein.

Preparation of the antigen emulsion: 0.8 cc. distilled water was pipetted into a 30 cc round bottomed flask, the flask was rotated rapidly on a flat surface while 0.9 cc 1 % cholesterol was pipetted into it from a 1.0 cc. pipette. The rotation was then continued for another 15 minutes more. 0.1 cc. of the antigen was added to the flask with a 0.1 cc or 0.2 cc. pipette, the pipette was blown empty, a glass stopper was placed in the flask and it was shaken vigorously for one minute; 2.5 cc. of physiological saline was added and the flask shaken vigorously for one minute; 3.0 cc was transferred from the flask into a round bottomed centrifuge tube which was then kept in a 56° C waterbath for 15 minutes. From the waterbath the tube was transferred to a centrifuge where it was rotated at 1,800 revolutions per minute for 15 minutes. After centrifugation the turbid supernatant fluid was discarded, the walls of the tube wiped with cotton or gauze, and then 3.0 cc of physiological saline were added to the tube. When the tube was gently shaken the sediment on the bottom of the tube was resuspended in this solution, which was used as the final antigen emulsion. A fresh emulsion was always prepared for each day.

Reaction technique: A hypodermic needle gauge 12 was used for dropping the antigen emulsion, in such a way that size of a drop was 1/120 cc. In other respects the performance and reading fully correspond with those of the VDRL. Clumps are smaller for this reaction than in the VDRL.

The Sitolipin Slide Test (Sitol.):

Antigen: The antigen was received from Professor Eero Uroma of the State Serum Institute, together with the necessary buffer solution. The antigen contained 0.05 % sitolipin, 0.3 % lecithin and 0.9 % cholesterol. The buffer was the same as in the VDRL. The preparation of the antigen emulsion was similar to that for the VDRL except that the larger buffer addition was replaced by an equal amount of 1 % saline. Moreover, the prepared emulsion was allowed to stand for one hour at least in a small test tube, from which the upper part only was taken into a syringe and used as antigen, the residue from the lower part of the tube being discarded.

Reaction technique: The performance of the reaction in all its phases corresponded with that of the VDRL.

The Berger Slide Test (Berger):

Thea reaction was carried out according to BERGER's (1943) original method.

Antigen: To ten parts of the Kahn antigen extract used one part of the British Pharmacopoeia tinct. benz. comp. was added. For each 10 cc of this solution 17 mg of Victoria blue was added. The solution obtained keeps at room temperature. The antigen emulsion was prepared separately for each day. 0.1 cc of antigen was pipetted into the bottom of a tube and onto this 1 cc of physiological saline solution was rapidly poured. The emulsion obtained must be shaken before use.

Reaction technique: The sera were inactivated as in the WR. 0.05 cc of each serum was pipetted into a paraffin ring, and an equal amount of antigen emulsion was dropped onto it from a 1 cc pipette. The glasses were then rotated as in the VDRL, for 4 minutes, after which the macroscopic reading of the reactions followed immediately.

Reading of the reaction: To indicate the degree of flocculation the symbols + + +, + + and + were used. + + + indicated a few, large, blue-black clumps in an otherwise clear medium; + + indicated smaller, but more numerous blue-black clumps in a clear medium: + indicated still smaller and denser clumps, where the medium could no longer be considered entirely clear. As controls were used the results obtained for known positive and negative sera. In negative sera the reaction result consisted of a blue, homogeneous, opaque emulsion only. The final results were reported in this reaction also as: +, ±, and —, where the degree of flocculation + + + and + + were given as + and the degree of flocculation +, as ±.

On a number of the positive sera the Kahn, VDRL, Rein — Bossak, Sitol. and Berger were also made quantitatively. The sera were diluted with physiological saline solution in the ratios 1:2, 1:4, 1:8, 1:16, etc. on which dilutions each reaction was then made by the same method as for the qualitative reaction. The dilution which still yielded the degree of flocculation + was entered as the titer.

Meinicke's Second Clarification Reaction (MCRII):

Antigen: The basic solution produced by AB Astra, Södertälje, containing beef heart muscle lipids, balsam of Tolu and Victoria blue was used. The antigen basic solution keeps at room temperature if protected from light.

Preparation of the antigen emulsion: 10 cc of 3.5 % saline solution was pipetted into a tube and into another similar tube, 10 cc of 3.5 % saline solution to which 0.01 % sodium bicarbonate had been added. Both tubes were then placed in a 56° C waterbath. 1 cc of the antigen basic solution was pipetted onto the bottom of each of two large test tubes and these tubes were also placed in a 56° C waterbath. When all four tubes had reached the temperature of the waterbath, the 3.5 % saline solution was rapidly poured into one of the tubes containing antigen basic solution and into the other the 3.5 % saline solution to which the sodium bicarbonate had been added. The antigen

emulsion containing sodium bicarbonate was immediately ready for use, the sodium bicarbonate-free antigen emulsion was kept in the waterbath for 2 minutes after the mixing. It was then ready for use. For each day fresh emulsions were prepared.

Sera were used uninactivated in this reaction.

Reaction technique: The reaction was made in two test tubes of which one belonged to the "main" series and the other to the sodium bicarbonate series. Into the main series tube was pipetted 0.2 cc and into the tube of the sodium bicarbonate series 0.1 cc of the serum to be tested. 0.5 cc of sodium bicarbonate-free antigen emulsion was then pipetted onto the serum in the main series tube, and 0.5 cc of antigen emulsion containing sodium bicarbonate onto the serum in the sodium bicarbonate series. The tubes were shaken by hand until the contents were well mixed, then left to stand at room temperature until the following day, when the reading of the reaction took place.

Reading of the reaction: In syphilitic sera the antibodies and the specific lipids of the antigen emulsion inter-react forming clumps to which the balsam of Tolu particles also adhere. These clumps sink to the bottom in about 16 hours and the solution above becomes clear, while in healthy sera it remains an opaque, grey-blue emulsion. To indicate the degree of clarification the symbols + + + +, + + +, + + and + were used. Where the main series tube was turbid, but the sodium bicarbonate series tube partly or totally clarified the symbol + + + + was used. This occurred only with sera rich in reagins, and hence a kind of inhibition phenomenon was involved. + + + indicated a result in which both tubes were clarified. + + indicated that the main series tube was clear, but the sodium bicarbonate series tube only partly clear. + indicated that the main series alone was clarified.

When comparing these results with the results reported for the flocculation reactions listed above the results + + + +, + + + and + + were taken as + (positive), the + results as ± (weakly positive) and the remainder as negative, even if there was some slight clarification in the main series tube. Because of the distinctness of the reaction control sera were considered unnecessary. The MCR11 was not carried out quantitatively.

Observations on the WR, Chol. WR and Kahn Based on the Material for 1946 — 50

Method of Dealing with the Material

The material comprises 126,344 blood samples on all of which the Kahn was made and on 79,716 samples on which the WR and chol. WR were also made.

In the study of the specificity of the reaction results the following criteria have been used:

— All positive results from patients at the Clinics of the University of Turku and in the General Hospital of Pori were considered to be caused by syphilis, if the journals, either in the medical history or condition showed anything indicative of syphilis.

— The positive results deriving from the suspected syphilis material in which were included the samples sent by Venereal Disease Outpatients Clinics, by specialists in venereal diseases and by prisons, were considered as specific, even where no control investigation had been made and no certain diagnosis mentioned.

— All the positively reacting samples sent by official physicians whose delivery note gave, without a question mark, a diagnosis indicative of syphilis, were also considered as specific reaction results.

— Where the delivery notes of the samples sent by general practitioners and advisory centers gave no certain diagnosis, or where the diagnosis mentioned no disease which, according to general experience, is known to produce biologic false positive seroreactions for syphilis, yet the reaction or reactions in control examinations proved to be continually positive, the positivity was counted as specific.

— All the samples the reactions of which could not be counted as specific according to the above criteria, were noted down as false positive results irrespective of the results of possible control examinations. As the control examinations generally came only after several days or weeks, it was not possible to separate technical errors into a distinct group.

These criteria for the definition of the specificity of a positive result are incomplete in many respects and contain many potential errors. However, due to the retrospective character of the observations made and the relatively large material it was not within the limits of the author's powers to check the reactions in detail by approaching the senders of the samples.

All the patients who gave positive samples were catalogued and only the first sample in the material in which some reaction was positive was subjected to examination. After the reaction results considered false positive on the above grounds had been eliminated, the material covered a total of 3,877 syphilities. This syphilis material also serves as the basis for the observations concerning the seropositive syphilis frequency and the diseases of seropositive syphilities to be discussed later.

Average Sensitivity of the WR, chol. WR and Kahn

The following Table shows in how many cases these three different reactions yielded positive results when the blood sample of each syphilitic first appeared in our material. On all these samples all three reactions were carried out. The percentages were calculated on the total of the seropositive syphilities in our material, numbering 3,877, and thus they do not indicate the sensitivity of the reactions in all syphilis cases. It has not been possible to ascertain how many syphilities are actually included in the material studied when all the cases which become seronegative when using these reactions are taken into consideration.

Table 1.

Average Sensitivity of the WR, chol. WR and Kahn in 1946 — 50.

	WR	Chol. WR	Kahn
Positive cases, total	2101	2844	3700
Percentage	54.2	73.4	95.4

Average Sensitivity of the WR, chol. WR and Kahn in Different Years

When studying in literature the percentages of the sensitivity of the same syphilis seroreaction obtained by different investigators, one cannot help observing the immense divergencies in the findings of different investigations. In order to find out to what extent the fact that the investigations have often been made at very different times may possibly have influenced the variation in the results, the average sensitivity of the WR, chol. WR and Kahn in different years has been studied during the period 1946 — 1950. The results are shown in Table 2, and Figure 1 shows them in diagrammatic form. The exclusively Kahn-positive have also been included as a separate group. The percentages have been computed on the total amount of positive cases for each year.

Table 2.

Average Sensitivity of the WR, chol. WR and Kahn in Different Years

Year	1946	1947	1948	1949	1950
WR-positive cases	663	476	347	367	248
Percentage	62.4	67.1	61.5	47.4	32.4
Chol. WR-positive cases	839	617	459	518	411
Percentage	78.9	87.0	81.6	66.8	53.7
Kahn-positive cases	1035	683	526	725	731
Percentage	97.4	96.3	92.9	93.6	95.4
Exclusively Kahn-positive cases	215	89	92	229	343
Percentage	20.2	12.6	16.3	29.6	44.8
Total positive cases	1063	709	564	775	766

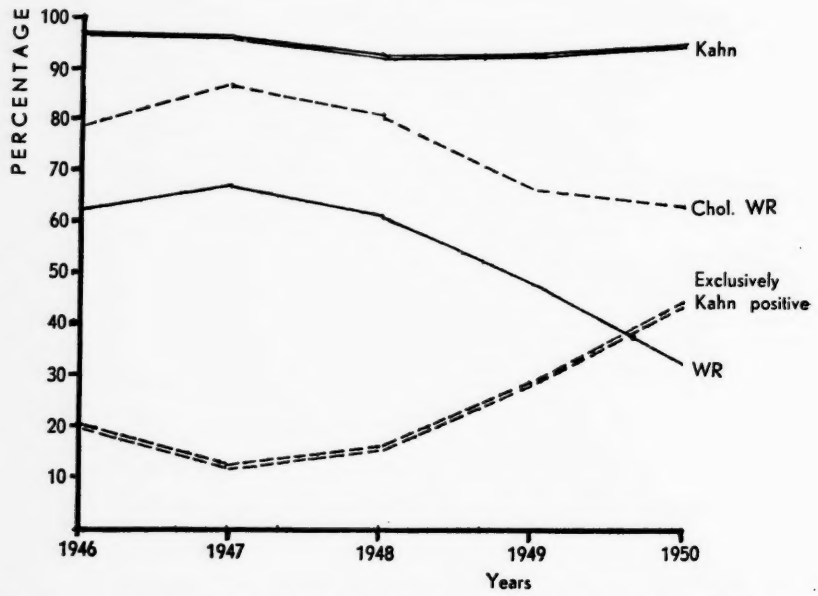


Figure 1. Average Sensitivity of the WR, chol. WR and Kahn in Different Years in diagrammatic form

From the results it is clearly seen, in the author's opinion, to what a great extent the percentages representing the sensitivity of the WR and chol. WR depend on the time when the investigations were made. Accordingly, serologic investigations carried out at

different times cannot be directly compared with each other. In Finland the number of reported new syphilis cases toward the end of World War II, reached its peak in the years 1946 — 47 and has been continually decreasing ever since (PUTKONEN 1950). The serologic results according to the present material conform with the fluctuations of these officially reported fresh cases. The syphilis epidemics are visible in the WR and chol. WR curves. With the decrease of syphilis and the treatment of old cases, the WR and chol. WR positivity is reduced, whilst the therapeutic measures only later affect the Kahn-positivity (*e.g.*, OLIN 1935) and thus when the serologically examined syphilis material is mainly composed of treated cases, the flocculation reactions — in our own material the Kahn, primarily, — will be the principal indicator of seropositivity. If we examine in Figure 1 the WR, chol. WR and Kahn curves only, we could, in the author's opinion, very well take them as representing the progress of these reactions in *o n e patient* with fresh syphilis under antisyphilitic treatment — the WR and chol. WR gradually grow negative or weaker while the Kahn stubbornly remains positive. *In general it may be stated that the combination of three reactions in use in our laboratory — the WR, chol. WR and Kahn — is chosen successfully in so far as, in the author's opinion, it simultaneously acts as indicator of both the activity of the disease and to a great extent also of its seropositivity. These three reactions are actually a kind of quantitative titration from which, for example, the effect of the treatment can be directly followed.*

Incidence of the WR, chol. WR and Kahn as the Only Positive Reaction

Table 3 shows the extent to which the three reactions used have appeared in our material as the only positive reactions. The percentages have been calculated on the total amount of positive results yielded for each of the reactions.

Table 3.

Incidence of the WR, chol. WR and Kahn as the Only Positive Reaction

	WR	Chol. WR	Kahn
Number of cases	18	88	985
Percentage	0.9	3.1	26.6

The results revealed by the Table are analogous with the results presented in the previous chapter. They show clearly that the sector of seropositivity obtained for the Kahn is considerably more extensive than that obtained for the WR.

Frequency of Weakly Positive Results

The larger the number of weakly positive results, difficult of interpretation, yielded by a reaction the more it hampers the use of that reaction in large-scale serodiagnostics. Table 4 shows the weakly positive results (±) in the present material for each of these reactions. The percentages have in this case too been calculated on the total amount of positive results for each reaction.

Table 4.

The Proportion of Weakly Positive (±) Results of the WR, Chol. WR and Kahn Positive Results

	WR	Chol. WR	
Number of cases	172	212	346
Percentage	8.2	7.5	9.4

In this respect it may be seen that there appears to be no great difference between these reactions in our material. The percentage appears rather low and can hardly be much reduced from what it is now. It is probable that if, for example, the Kahn were sensitized by some means in order to make the weak reactions stronger, then it might conceivably extract an equal quantity of quite new weakly positive reactions. On the basis of the present material it seems, then, as if this margin of weakly positive reactions were largely the same for the different reactions.

Incidence of False Positive Results

When describing the method of dealing with the material the author also presented the bases on which false positive results were determined. The distribution of these false results between the different reactions is given in Table 5. For reference the total

number of reactions performed in each reaction has been used. As mentioned before, it was not feasible to separate technical errors as a special group.

Table 5.

Distribution of False Positive Cases between the WR, Chol. WR and Kahn

	WR	Chol. WR	Kahn
Number of false positive cases ..	11	38	140
Total of cases tested	79716	79716	126344
Percentage	0.014	0.048	0.11

The quantity of false positive results in the material is noticeably low. However, it agrees well with the corresponding figures of MAIJALA's (1942) material (9,104 patients in the Medical Outpatients Clinic) which for the relevant reactions were as follows: WR 0.04 %, chol. WR 0.06 % and Kahn 0.11 %. In the control material of ANTTONEN (1945), covering 917 patients in various hospitals, however, a much larger quantity of false positive results appeared. He obtained the following percentages: WR 3, chol. WR 4 and Kahn 6. The percentages of false positive reactions obtained by Finnish investigators for these reactions vary within these limits. In general it may be observed that earlier investigators reported more of these false reactions than has appeared in the investigations of recent years (PÄTIÄLÄ 1950).

In order to show which diseases or conditions were the most important causes of false positive reactions in our material Table 6 gives a distribution of the 65 cases in which, with some certainty it could be considered that a biologic false positive reaction was in question. The appearance and disappearance of the positive reaction coincident with some disease or condition was used as the criterion.

As in other statistics for the Northern Countries mentioned earlier, various diseases of the lungs and respiratory tract hold the leading position. Individual cases of false positive results of this kind were caused by diseases like endocarditis lenta, lupus erythematodes, paratyphoid, poliomyelitis, rheumatic fever, etc., to give only a few examples. That is to say, various infectious diseases were chiefly concerned.

Table 6.

Distribution of the Most Important Causes of Biologic False Positive Reactions in 65 cases

Diseases or Condition	Number
Pneumonia	13
Bronchitis	3
Pleuritis	3
Tub.pulm.	4
Erysipelas	5
Hepatitis ac.	5
Myelitis, Meningitis	3
Diabetes mellitus	2
Malaria	2
Narcosis (ether)	2
Morbi varior. (individual cases)	23
Total	65

Table 7 gives the child patients selected from among the above false positive cases. Originally there were in our material 35 children reacting positively for one or more reactions. The information available on 4 of them was insufficient, 23 were congenital syphilities and 8 cases proved to have yielded a false positive reaction. In the parents of these 8 children no signs of syphilis could be shown.

Table 7.

Child Patients Giving False Positive Results

Disease	Age	Serologic Status		
		WR	Chol. WR	Kahn
Pneumonia	9 years	—	+	—
Osteogenesis imperfecta	7 years	—	±	±
Bronchopneumonia	4 years	—	±	—
No objective findings	4 years	—	—	+
Diabetes mellitus	3 years	—	+	—
Tuberculous meningitis	2 years	—	—	+
Pulmonary tuberculosis	10 months	—	+	±
Bronchitis and enteritis	3 months	±	+	±
		1	6	5

Thus about $^1/_4$—$^1/_5$ of the positive reactions in these child patients in this material proved to be false positive. These false positive reactions were obtained most often for the chol. WR and Kahn.

Summary of Observations on the WR, Chol. WR and Kahn

The author has analysed the WR, chol. WR and Kahn material of 1946 — 50 of the Department of Serology and Bacteriology of the University of Turku. This material comprises 126,344 blood samples tested by the Kahn reaction and of 79,716 samples tested by WR and chol. WR in addition to the Kahn. The cases yielding positive results were catalogued and only the first positive sample was taken into consideration. After the cases considered as false positive reactions had been eliminated, 3,877 seropositive syphilis cases remained.

Of the 3,877 syphilis cases considered as seropositive on the basis of the reactions studied, an average of 54.2 % were WR-positive, 73.4 % chol. WR-positive and 95.4 % Kahn-positive.

When examining the average sensitivities of these reactions in different years, the author observed that the percentage of the Kahn-positive cases had remained nearly the same during all the five years, the WR- and chol. WR-positive, however, showed a continuous, considerable decrease since the year 1947, while the number of only Kahn-positive cases increased from 12.6 % to 44.8 %. The author, examining this phenomenon, came to the conclusion that syphilis epidemics are visible in the first place in the WR and chol. WR positivity curves. In other words, they indicate the proportion of active, untreated or slightly treated syphilis in the material of each period.

The WR was the only positive reaction in 0.9 % of the total positive results yielded for it. For the chol. WR the corresponding figure was 3.1 and for the Kahn, 26.6 %. Broadly speaking, all three reactions yielded an equal number of weakly positive reactions.

The percentages showing the occurrence of false positive results were 0.014 for the WR, 0.048 for the chol. WR and 0.11 for the Kahn. Most prominent among diseases causing false positive re-

actions were diseases of the lungs and respiratory tract, followed by erysipelas, acute hepatitis, myelitis and meningitis, etc.

Of the 35 positive results obtained from children, 8 proved to be false positive reactions.

The Cardiolipin and Sitolipin Antigens in the Flocculation Reaction

Material and its treatment

As has been previously mentioned two cardiolipin slide tests (the VDRL and Rein — Bossak) and one sitolipin slide test were used in the present investigation. With these reactions a total of 7,928 sera were investigated. As control reactions the WR, chol. WR and Kahn were made for all the sera, 4,415 sera were also tested by the Berger and 4,260 sera by the MCR II.

The sera examined were received from the following persons or institutions:

Medical Clinic, University of Turku	928 sera
Surgical Clinic, University of Turku	972 „
Gynecologic-Obstetrical Clinic, University of Turku	689 „
Dermato-Veneralogical Clinic, University of Turku	191 „
Pediatric, Oto-Rhino-Laryngological and Ophthalmological Clinic, University of Turku	380 „
General Hospital, Pori	415 „
Insane Asylums (Turku, Halikko, Harjavalta, Uusikaupunki, Seili, Insane Asylum for Prisoners)	269 „
Other Hospitals	341 „
Outpatient Clinics for Venereal Diseases in Turku and Pori	249 „
General Practitioners	1,202 „
Advisory Centers	1,033 „
Prisons	110 „
Factories, Blood Service, Poor Law institutions	1,149 „
Total	7,928 sera

Of all cases yielding positive results for one or more reactions a card index was kept. Information on hospital patients in the clinics of the University of Turku was checked by the author from hospital journals. General practitioners, venereal disease outpatient clinics, rural hospitals, physicians at advisory centers and prisons, were sent a questionnaire on each positive case including the following questions about the patient:

Has syphilis ever been diagnosed in the patient and, if so, when?

What was the stage of the disease at the moment of the diagnosis (Syphilis I, Syphilis II, latent Syphilis, Syphilis III in its various forms, Congenital Syphilis, unknown)?

How many combined (Bi + Neos) courses of treatment has the patient been given and how many units of penicillin?

If there is no syphilis in the medical history, are there any symptoms indicative of syphilis (pupils, patellar reflexes, condition of heart, a possible cerebrospinal fluid finding, abortions, signs of congenital syphilis)?

If it is evident that syphilis is not concerned, has the patient any disease which might be the cause of possible false positive reaction (pneumonia, bronchitis, pleuritis, erysipelas, malaria, scarlatina, mononucleosis infectiosa, hepatitis, recent vaccinations, etc.)?

In addition a control sample was requested.

If the required information was not received after two requests, the positively reacting case concerned was transferred to the group of undetermined cases.

Defects in the Method of Treating the Material: It was not feasible for the author to perform the clinical examination of the positively reacting cases personally. Evaluations had to be based on the journals and information received through questionnaires. As the examinations of the patients were made by several physicians, the data obtained are not homogeneous in quality. The physicians in more rural conditions often had no facilities for special examinations (cerebrospinal fluid, Roentgen etc.); their personal knowledge of the patients, however, was an advantage. The supply of control samples was meager in spite of requests and most of them arrived only after some weeks, when their control value was already questionable. Thus technical errors could not be separated as a special group.

Average Sensitivity of the Reactions Employed

Owing to the fact that sera to be tested were not invariably sufficient for all 8 reactions, the material had to be divided into 4 groups within which simultaneous tests were effected. Table 8 gives these groups and the results obtained in each group. The positivity percentages have been computed on the total amount of the positively reacting sera.

Table 8.

Average Sensitivity of the Reactions Employed in the Sub-Groups of the Total Material (7,928 sera)

Group		Positive sera			All sera		
		Positive	Negative	Positivity %	Positive	Negative	Total
I	WR	56	359	13.5	415	1852	2267
	Chol. WR	103	312	24.8			
	Kahn	349	66	84.1			
	VDRL	300	115	72.3			
	Rein — Bossak	314	101	75.7			
	Sitol.	313	102	75.4			
II	WR	26	155	14.4	181	1065	1246
	Chol. WR	43	138	23.8			
	Kahn	131	50	72.4			
	VDRL	135	46	74.6			
	Rein — Bossak	137	44	75.7			
	Sitol.	141	40	77.9			
	MCR II	112	69	61.9			
III	WR	52	306	14.5	358	1043	1401
	Chol. WR	91	267	25.4			
	Kahn	282	76	78.8			
	VDRL	231	127	64.5			
	Rein — Bossak	218	140	60.9			
	Sitol.	219	139	61.2			
	Berger	290	68	81.0			
IV	WR	73	481	13.2	554	2460	3014
	Chol. WR	113	441	20.4			
	Kahn	360	194	65.0			
	VDRL	292	262	52.7			
	Rein — Bossak	295	259	53.2			
	Sitol.	272	282	49.1			
	Berger	418	136	75.5			
	MCR II	320	234	57.8			
					1508	6420	7928

Table 9 gives the mean value of the positivity percentage of each reaction computed on the basis of these four investigation series.

Table 9.

Average Value of the Positivity Percentage of the Reactions Employed computed on the Value of Four Different Serum Series

WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
13.9	23.6	75.6	66.0	66.4	65.9	78.3	59.9

Occurrence of the Reactions Employed as the Only Positive Reaction

Table 10 shows the numbers of the cases in which each reaction alone was positive.

Table 10.

Occurrence of the Reactions Employed as the Only Positive Reaction

WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
0	1	139	3	8	14	112	37

In addition it should be mentioned that in 90 cases the VDRL, Rein-Bossak and Sitol. were either all three together or at least two of them the only positive reactions.

Average Sensitivity of the Reactions Employed in the Different Stages of Syphilis

Because new syphilis cases in the district served by this laboratory are very few in number at present, no fresh syphilis cases could be included in the material. Therefore the positive material has been grouped as follows: Latent Syphilis, Tertiary Syphilis, Congenital Syphilis. The material, it is true, comprises 29 positive samples from 18 patients who, according to the physician who sent the samples, had been in the primary stage of syphilis at the

moment of diagnosis. However, as all these 18 patients had been under specific treatment for varying periods with continued seropositivity, it was not considered a great error in the clinical sense to place them in the latent syphilis group. On the same principle the author has included in this group the 46 positive samples from 27 patients whose disease had been diagnosed as in the secondary stage, but who had all received specific treatment. No patients in either group had any symptoms on the basis of which they could still have been considered to be in the primary or secondary stage.

In the tertiary syphilis — neurosyphilis — group are included all patients suffering from cardiovascular syphilis, the cases of syphilis cerebri, syphilis cerebrospinalis, dementia paralytica and tabes dorsalis as well as the patients with tertiary skin or internal organ manifestations not included in the forms listed above.

Latent Syphilis: The material comprises 582 samples from 365 patients. The results are seen from Table 11. As the Berger and MCR II were not carried out on all samples, no positivity percentage could be computed for them. The positivity percentage has been computed on the total amount of positively reacting specimens.

Table 11.

Distribution of Positive Results between the Reactions Employed in the Latent Syphilis Group.

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	116	204	525	488	481	471	267	228
Positivity %	19.9	35.1	90.2	83.9	82.6	80.9		
Negative	466	378	57	94	101	111	47	41
Lacking	—	—	—	—	—	—	268	313
Total	582	582	582	582	582	582	314	269

Of these samples 500 were derived from 319 treated latent syphilis patients, the remaining 82 samples were taken from 46 untreated latent syphilis patients.

Tertiary Syphilis and Neurosyphilis: The material comprises 166 samples from 119 patients. The results can be seen from Table 12.

Table 12.

Distribution of Positive Results between the Reactions Employed in the Tertiary Syphilis — Neurosyphilis Group

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	47	79	155	148	150	150	78	60
Positivity %	28.3	47.6	93.4	89.2	90.4	90.4		
Negative	119	87	11	18	16	16	8	4
Lacking	—	—	—	—	—	—	80	102
Total	166	166	166	166	166	166	(86)	(64)

86 of these 119 patients had been under specific treatment, 19 had not been treated at all and no information was obtained about the possible treatment of 14 patients.

Congenital Syphilis: The material comprises 41 samples from 28 patients. The results are given in Table 13.

Table 13.

Distribution of Positive Results between the Reactions Employed in the Congenital Syphilis Group

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	10	12	38	30	29	27	19	17
Positivity %	24	30	93	73	70	66		
Negative	31	29	3	11	12	14	6	2
Lacking	—	—	—	—	—	—	16	22
Total	41	41	41	41	41	41	(25)	(19)

23 of the patients in this group received specific treatment, 3 were untreated and no information was obtained about the possible treatment of 2 patients.

All Certain Syphilis Sera: In order to form a general idea of the sensitivity of the reactions employed in all syphilis cases the results of Tables 11, 12 and 13 are combined in Table 14. Thus the material comprises 789 samples from 512 syphilis patients.

Table 14.

Distribution of Positive Results between the Reactions Employed in the Total Syphilis Material

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Number of sera examined	789	789	789	789	789	789	425	352
Positive sera included in the above	173	295	718	666	660	648	364	305
Positivity %	21.9	37.4	91.0	84.4	83.7	82.1	(85.6)	(86.6)

Doubtful Syphilis Cases: The present material included 73 patients, whose medical history contained nothing to indicate syphilis. As 65 of them had either in the medical history or condition one or more factors which in addition to a positive seroreaction or seroreactions seemed indicative of syphilis, they were counted as doubtful syphilis cases. In this group are also included 8 cases which, due to some other disease, had been given penicillin treatment, because it was no longer possible to judge their condition. From the members of this group, numbering 73, a total of 115 samples was taken. The serologic results obtained can be seen from Table 15.

Table 15.

Distribution of Positive Results between the Reactions Employed in Doubtful Syphilis Cases

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	7	9	73	56	60	65	58	30
Positivity %	6.1	7.8	63.5	48.7	52.2	56.5		
Negative	108	106	42	59	55	50	19	39
Lacking	—	—	—	—	—	—	38	46
Total	115	115	115	115	115	115	(77)	(69)

Undetermined Cases: In spite of repeated inquiries there remained in the material 299 sera reacting positively for one or more reactions, on which sufficient information to make it possible to confirm the specificity or possible non-specificity of the

positive results was not available. The serologic results obtained in this group can be seen in Table 16.

Table 16.

Distribution of Positive Results between the Reactions Employed in Undetermined Cases

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	32	41	188	147	153	134	142	60
Negative	267	258	111	152	146	165	67	117
Lacking	—	—	—	—	—	—	90	122
Total	299	299	299	299	299	299	(209)	(177)

Effect of Antisyphilitic Treatment on the Average Positivity of the Reaction Employed

In studying the effect of the antisyphilitic treatment given to the patients only the latent syphilis group in the material has been considered, because the other groups would have been too restricted for this purpose.

On the basis of the quantity and quality of the antisyphilitic treatment given to the patients the material was divided into the following five groups:

1. Untreated cases (Table 17): The group comprises a total of 82 sera. The patients from whom the samples had been collected had received no specific treatment.

2. Treated cases, Group A (Table 18): The group comprises 325 sera. The patients in this group had been given $\geqq 4$ courses of combined treatment (this course of treatment is that used in Finland and usually given over a period of 5 weeks and comprising 10 bismuth and 10 neosalvarsan injections. The most commonly used doses were 0.2 g Bi + 0.6 g neos.) or $\geqq 6$ million I.U. of penicillin.

3. Treated cases, Group B (Table 19): The group comprises 91 sera. The patients in this group had been given < 4 courses of combined treatment or < 6 million I.U. of penicillin. Those treated exclusively with Bi are also included in this group.

4. Cases chiefly treated with penicillin (Table 20): The group comprises 64 sera. The patients in this group had been given $\geqq 6$ million I.U. of penicillin but only $\leqq 2$ courses of combined treatment or no combined treatment at all.

5. Cases treated chiefly with the combination of neosalvarsan and bismuth (Table 21): The group comprises 175 sera. The patients in this group had been given $\geqq 4$ courses of combined treatment but only $\leqq 1{,}2$ million I.U. of penicillin or no penicillin at all.

The results of the effect of the antisyphilitic treatment of varying degrees on the average positivity of the reactions used are shown by the Tables 17—21.

Table 17.

Distribution of Positive Results between the Reactions Employed in Untreated Latent Syphilis Cases

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	35	50	79	72	69	71	42	30
Positivity %	43	61	96	88	84	87		
Negative	47	32	3	10	13	11	9	4
Lacking	—	—		—		—	31	48
Total	82	82	82	82	82	82	(51)	(34)

Table 18.

Distribution of Positive Results between the Reactions Employed in Treated Latent Syphilis Cases. Group A

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	51	97	294	267	266	265	145	135
Positivity %	15.7	29.8	90.5	82.2	81.8	81.2		
Negative	274	228	31	58	59	60	33	27
Lacking	—	—	—	—	—	—	—	
Total	325	325	325	325	325	325	(178)	(162)

Table 19.

Distribution of Positive Results between the Reactions in Treated Latent Syphilis Cases, Group B

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	23	31	85	80	77	70	45	30
Positivity %	25	34	93	88	85	77		
Negative	68	60	6	11	14	21	7	6
Lacking	—	—			—		39	55
Total	91	91	91	91	91	91	(52)	(36)

Table 20.

Distribution of Positive Results between the Reactions Employed in the Latent Syphilis Cases Treated Chiefly with Penicillin

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	21	35	58	56	56	56	36	32
Positivity %	33	55	91	88	88	88	5	
Negative	43	29	6	8	8	8		6
Lacking	—		—	—	—	—	23	26
Total	64	64	64	64	64	64	(41)	(38)

Table 21.

Distribution of Positive Results between the Reactions Employed in the Latent Syphilis Cases Treated chiefly with a Combination of Neosalvarsan and Bismuth

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Positive	13	31	155	132	133	132	72	74
Positivity %	7.4	17.7	88.6	75.4	76.0	75.4		
Negative	162	144	20	43	42	43	23	17
Lacking	—	—	—	—	—	—	80	84
Total	175	175	175	175	175	175	(95)	(91)

Results of Quantitatively Performed Flocculation Reactions

A total of 380 positive sera were investigated quantitatively with the two cardiolipin slide tests and the sitolipin slide test; on a number of these sera the quantitative Berger and Kahn were also made. However, only the quantitatively examined sera in the treated and untreated latent syphilis and tertiary syphilis groups, a total of 248 sera, will be discussed in detail below. Of the remaining quantitatively examined sera the majority falls into the undetermined positive cases; therefore it is not considered relevant to report on them. As the material was studied retrospectively, it appeared, unfortunately too late, that only few quantitative examinations had involved a serum yielding a false positive result, because the quantitative examinations had by force of circumstances to be made only of samples which were sufficiently large for titrations requiring much serum. This group cannot, therefore, be taken into consideration in the study.

The results of the quantitative examinations of the 248 positive sera are seen in Table 22. The degree of dilution is given in the Table as serum dilution, not as final reaction dilution.

Table 22.

Results of Quantitative Investigations in the Treated Latent Syphilis, Untreated Latent Syphilis and Tertiary Syphilis Group

Reaction	VDRL									Rein—Bossak										Sitol.										Berger										Kahn										
Dilution	Undil.	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	Undil.	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	Undil.	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	Undil.	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	Undil.	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256		
Number of sera	47	66	16	16	9	4	4	0	0	41	45	36	20	8	7	2	3	0	64	54	18	14	5	5	2	0	0	28	20	9	9	1	2	0	0	0	39	39	31	13	9	1	1	1	0	Treated Latent Syphilis
Total sera investigated	162									162									162									69									134									Treated Latent Syphilis
Number of sera	5	8	7	7	3	2	0	0	0	5	5	8	3	8	0	3	0	0	5	7	5	8	5	2	0	0	0	3	2	3	5	1	0	0	0	0	4	7	4	7	2	1	3			Untreated Latent Syphilis
Total sera investigated	32									32									32									14									28									Untreated Latent Syphilis
Number of sera	8	13	17	10	2	3	1	0	0	8	5	17	11	6	5	1	0	1	11	14	10	9	5	3	2	0	0	6	3	6	1	1	0	1	1	1	7	8	13	7	4	0	3	2	0	Tertiary Syphilis
Total sera investigated	54									54									54									20									44									Tertiary Syphilis

The results given in Table 22 are presented in graph-form in Fig. 2.

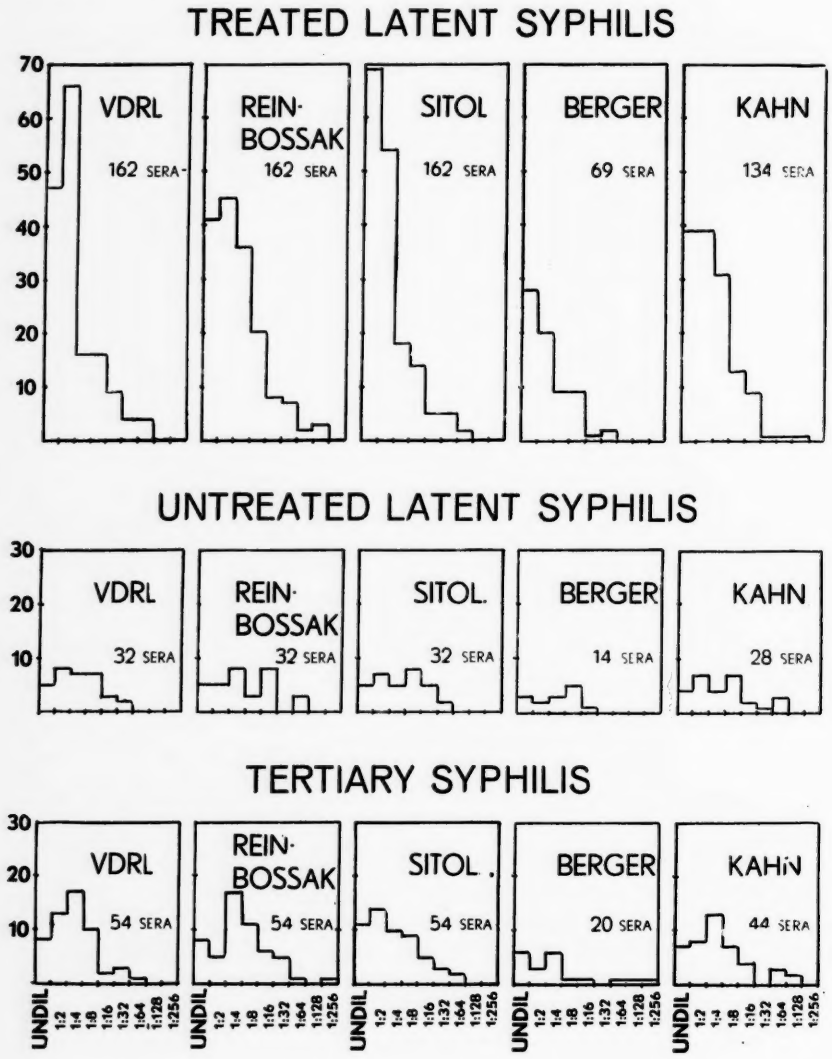


Figure 2. Results of Quantitative Investigations in the Treated Latent Syphilis, Untreated Latent Syphilis and Tertiary Syphilis Groups Present in Graph-form

False Positive Reactions

False positive results were obtained for 295 sera, derived from 220 patients. In table 15 an attempt is made to present these cases as briefly as possible. None of the patients presented in the Table had either anamnestical or clinical signs of syphilis. The patients are presented in the order they appear in the card index and, for the sake of simplification, marked with current numbers only.

Table 23.

List of Patients with False Positive Seroreactions for Syphilis

No. of Patient	Date [1]	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
1		—	—	—	—	—	—	—	±	Pregnancy IX month.
2		—	—	—	+	+	+	±		Pregnancy VII month.
3	May 30	—	—	—	+	+	±	±	—	Pulm. tub.. Nephropathia.
	June 6	—	—	±	±	—	±	+	—	Schizophrenia.
4		—	—	—	±	—	+	+	—	Nil.
5		—	—	—	±	—	±	+		„
6		—	—	—	±	±	±	+	—	„
7		—	—	—	—	—	—	—	±	Kolpitis (candida albicans). Hypomenorrhea.
8		—	—	—	±	±	±			Hypertonia. Blood donor (several times).
9		—	—	—	—	—	±	—	—	Bronchitis. Manic-depressive Psychosis.
10		—	—	—	±	±	±		—	Ca ventr.. Haematemesis. Blood transfusions given. Death.
11	April 24	—	—	+	+	+	—			Pregnancy IX month. 3 months earlier common cold. Biliary operation in 1949.
	May 7	—	—	±	+	+	±			
	May 12	—	—	+	+	+	—			
12	July 17	—	—	+	—	—	—	+		Pregnancy IV month. July 23 Kahn still +.
13	July 11	—	—	+	—	—	—	±	±	Fatigue.
	July 19	—	—	—	±	±	±	+	—	
14		—	—	—	—	—	—	±	—	Pregnancy VII month.
15		—	—	—	—	—	—	±	—	Post-pleurisy condition. Pregnancy.
16		—	—	—	—	+	±	—		Hypochromic Anaemia.
17		—	—	—	±	±	±			Neurosis gravis.

[1] Date is given only when control examinations have been made.

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
18		—	—	—	—	±	±			Pregnancy VI month.
19	June 27	—	—	—	—	—	—	±	—	Childbirth. Child asymptomatic.
	June 2	—	—	—	±	—	—	±	—	
20		—	—	—	—	±	+	—	—	Furunculosis.
21	May 18	—	—	+	—	—	—			Cystitis (e. coli). Anaemia.
	May 28	—	—	+	—	—	—	—		Eczema m.a. E.S.R. 15 (mm/1h).
22		—	—	+	—	—	—	+		Nil.
23		—	—	—	—	—	—	+	—	„
24	July 4	—	—	—	—	—	—	±	—	Lumbago (Myositis).
	July 11	—	—	—	—	—	—	±	—	
25		—	—	±	—	—	—	—	—	Nil.
26	May 4	—	—	+	+	+	+			Myodeg. et insuff. cordis. Anaemia of unknown origin. For abdominal observation.
	May 8	—	—	+	+	+	+			
27		—	—	±	—	—	—		—	Pregnancy VIII month. During pregnancy Kahn gradually became positive.
28		—	—	—	—	—	—	—	±	Acute Gastro-enteritis.
29		—	—	±	±	+	—	+	—	Pregnancy IV month.
30	June 25	—	—	±	+	—	—	—	—	Child. BCG vaccination April 11.
31		—	—	—	+	—	±	±	—	Nil.
32		—	—	—	—	—	—	±	—	„
33		—	—	—	—	—	—	±	—	Convalescence after influenza.
34	July 8	—	—	±	—	—	—	—	—	Pneumonia. Diabetes mellitus.
	July 11	—	—	—	—	—	—	—	—	
35		—	—	—	—	—	—	±	—	Nil.
36		—	—	—	—	—	—	±	—	Pregnancy VII month. 6 months earlier BCG and diptheria vaccinations.
37	Apr. 10	—	—	+	+	+	±		+	Pregnancy.
	May 8	—	—	+	+	—	—			
38	July 17	—	—	±	—	±	±	+	—	Ca bronchi. On July 20 Kahn still +.
39		—	—	+	±	±	±	±		Pyosalpinx l.s.
40	July 13	—	—	±	—	—	—	—	—	Pregnancy. During an earlier pregnancy Kahn had also once been +.
	July 20	—	—	—	—	—	—	—	—	
41		—	—	—	±	±	±	±	—	Pregnancy.
42	Apr. 28	—	—	±	—	—	—			Pregnancy VIII month.
	May 7	—	—	+	—	—	—			
	June 25	—	—	+	—	—	—	±	—	

No. of Patient	Date		WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
43	June	16	—	—	—	—	—	—	+		Pregnancy.
	June	22	—	—	—	—	—	—	—	—	
44	June	11	—	—	±	±	—	±	—		Pregnancy. Pruritus cutis. Vitamin B treatment.
45			—	—	—	—	—	—	+	—	Chronic nephritis. Retinitis albuminurica.
46			—	—	—	—	—	—	±	—	Gastric ulcer.
47			—	—	—	—	—	—	±	—	Hepatitis. Atrophia flava hepatis. Death.
48	June	19	—	—	—	—	—	—	+	—	Nil.
	July	18	—	—	±	—	—	—	+	—	
49			—	—	—	—	—	—	±	—	Pregnancy V month.
50			—	—	—	—	—	—	+	—	Nil.
51	July	12	—	—	—	—	—	—	±	—	Tumor pulm. sin.. Acute Nephritis.
	July	14	—	—	+	+	+	+	+	—	Aug. 3, Kahn again —.
	July	18	—	—	+	+	+	+	+	—	
52	July	5	—	—	—	±	±	+	±	—	Ca pancreatis c.metast. hepatis.
	July	10	—	—	±	+	+	+	—	—	Death.
53			—	—	—	—	—	—	—	±	Acute Appendicitis. Appendiectomy.
54	June	9	—	—	—	±	+	±	—		Tumor malign. ut. c.metast.
	June	19	—	—	—	—	—	—	—		Roentgen treatment.
55			—	—	—	—	—	—	±	—	Nil.
56	July	12	—	±	+	+	+	+	+	+	Pneumonia some months earlier, now asymptomatic.
	July	15	—	—	+	+	+	±	+		
	July	18	—	—	+	+	+	±	+		
57			—	—	—	±	±	±	—	—	Nil.
58			—	—	—	—	—	±		—	Active pulm. tub..
59	Apr.	17	—	—	—	+	+	+		±	Broncho-pneumonia. Hypertonia.
	Apr.	25	—	—	—	—	±	±			
60	Apr.	20	—	—	±	—	—	—	±		Myodeg. et insuff. cordis.
	Apr.	24	—	—	±	—	—	—			
	Apr.	27	—	—	+	—	—	—			
61	Apr.	26	—	—	±	—	—	—			Emphysema pulm.. Myodeg. et insuff. cordis. Hypertonia.
	May	4	—	—	—	—	—	—			
62	Apr.	27	—	—	+	±	±	±			Pulm. tub.. Myodeg. et insuff. cordis.
	May	2	—	—	+	+	+	+			
63	May	2	—	—	—	±	±	±			Pregnancy X month. Childbirth.
	May	15	—	—	—	—	—	—			
64	May	19	—	—	±	—	—	—			Acute Pneumonia.
	May	22	—	—	—	—	—	—			

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
65	Apr. 17	—	—	±	+	+	+		±	Hypertonia. Myodeg. et insuff.
	June 19	—	—	+	—	—	—	±	+	cordis.
66		—	—	±	—	—	—		—	Laceration of thumb and severing of tendon. Suture.
67		—	—	—	±	±	±			Pregnancy IV month.
68		—	—	—	±	+	±			Bronchitis. Entero-colitis subac..
69	May 16	—	—	+	+	+	+			Patient has earlier had Mor-
	May 18	—	—	+	—	—	+			billi, Typhus, Typhoid and
	May 29	—	—	+	+	+	+	+	+	Pleuritis. At present: Vulnus perf. sclerae, Prolapsus iridis.
70	July 18	—	—	±	—	—	—	—	—	5 months earlier Kahn —. Chro-
	July 28	—	—	+	—	—	—	—	—	nic Endocarditis ulcerosa (diagnosis on autopsy).
71	July 18	—	—	±	—	—	—	—	—	Fluor alb.. July 30 Kahn —.
72	July 7	—	—	+	—	—	—	±	—	Pregnancy VII month. July 18 Kahn still +.
73		—	—	—	—	—	—	±	—	Arteriosclerosis.
74	July 4	—	—	±	—	—	—	—		Diphtheria.
	July 7	—	—	—	—	—	—	±		
75		—	—	—	—	—	—	±		Pregnancy VII month.
76	June 19	—	—	±	—	—	—	+	±	I-para, Acute Nephrogestosis,
	June 21	—	—	±	—	—	—	—	—	child asymptomatic. Kahn — during pregnancy. In 1947 Parotitis.
77	June 8	—	—	—	±	±	+	±		Helminthiasis (Diphyllobotr.
	June 18	—	—	—	—	—	—	—	—	lat.).
78	June 5	—	—	—	—	±	—	±	—	Caesarean section (under ether
	June 12	—	—	—	—	±	—	—		narcosis), child healthy.
79	May 14	—	—	±	—	—	—			Pleuropneumonia l.dx. (Cold
	May 20	—	—	—	—	—	—			agglutination test 1: 128).
80		—	—	—	—	—	±	—	—	Neurosis.
81	May 2	—	—	—	—	±	±			Pregnancy.
	May 28	—	—	—	—	—	—	—	—	
82		—	—	±	—	—	±	±	—	Nil.
83		—	—	±	—	—	—	±	—	Sinusitis max. l.a., Sinusitis frontalis l.s..
84		—	—	—	—	—	—	±	—	Schizophrenia.
85		—	—	—	—	—	—	+	—	Schizophrenia.
86		—	—	±	—	—	—	+	+	Achylia gastr.
87	May 29	—	—	—	—	—	—	—	±	Leuchaemia myeloica.
	May 31	—	—	—	—	—	—	—	—	
88	July 4	—	—	—	±	±	—	—	—	Acute Psychosis.
	July 12	—	—	—	—	—	—	—	—	

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
89	Apr. 18	—	—	—	±	±	±		±	Hypochromic anaemia. Achylia gastr.. Hypertonia. Amblyopia.
	Apr. 26	—	—	—	—	—	—			
90		—	—	—	—	—	—	+	—	General asthenia. Psychosis (Often been in an asylum).
91	June 1	—	—	±	—	±	±	+	—	Schizophrenia. Status post contus. cerebri.
	June 11	—	—	—	—	—	—	—	—	
92	Apr. 25	—	—	—	—	±	±			Incipient Schizophrenia.
	May 5	—	—	—	—	—	—			E.S.R. 1.
93	May 18	—	—	+	—	—	—			Coliuria. Eczema m.a.. Paronychia dig. II ped. sin..
	May 28	—	—	+	—	—	—	—		
94		—	—	—	—	—	—	±		Nil. Menstruation.
95	Apr. 5	—	—	—	—	—	—	±	—	Manic-depressive Psychosis (mania).
	Apr. 12	—	—	—	—	—	—	±	—	
96		—	—	—	—	±	—			Schizophrenia.
97		—	—	—	—	—	—	±	—	Child. Enuresis.
98		—	—	—	—	±	±	±	—	Marasmus senilis. Gangraena senilis glutei.
99	May 9	—	—	—	—	±	±			Acute infection. Helminthiasis (Diphyllobotr. lat.), cerebrospinal fluid 0.
	May 16	—	—	—	—	—	—			
100	May 30	—	—	±	—	—	—	+	—	Struma nodosa toxica (PAD).
	June 8	—	—	—	—	—	—	+	—	Otitis media ac.l.dx.. Strumectomy.
101		—	—	—	±	±	±			Struma adenomatosa foetalis (PAD). Strumectomy.
102		—	—	—	—	—	—	±	±	Acute Appendicitis. Appendiectomy. Salpingo-oophoritis.
103	May 29	—	—	—	—	—	—	±	—	Sarcoma parietis thoracis
	June 8	—	—	—	—	—	—	±	—	(PAD). Pulm. tub., in 1946 pneumonia twice.
104		—	—	—	±	+	±	—	—	Exarticulatio traum. pollicis dx.. E.S.R. 3.
105	July 19	—	—	±	—	—	—	—	—	Concussion. E.S.R. 25.
	July 28	—	—	—	—	—	—	—	—	
106	June 19	—	—	±	—	—	—	±	—	Acute febrile Gastroenteritis. E.S.R. 91. Leucos. 17100. June 22 Kahn —.
107		—	—	+	+	+	+	±		Prolapsus disci intervertebr. praesacr. Cerebrospinal fluid: Pandy +, Nonne —. Leucos. 4 mm³, WR —, Chol. WR —.
108	June 8	—	—	+	—	—	—	+	+	Paratyphoid and Cholelithiasis
	June 8	—	—	+	—	—	—	+	+	(operated) 4 months earlier. Now pyelonephritis and Anaemia hypochr.. E.S.R. 66.

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein—Bossak	Sitol.	Berger	MCR II	Remarks
109		—	—	—	±	±	±			Salpingo-oophoritis bilater. et parametritis. E.S.R. 72.
110		—	—	—	—	—	—	+	—	Operation for prolapsed urethra. E.S.R. 38.
111		—	—	—	—	±	±	—		Ca recti.. E.S.R. 25.
112		—	—	—	—	—	—	±	—	Operation for prolapsed urethra. Myocardial Infarction. Death. E.S.R. 27.
113	June 27	—	—	+	+	±	±	+	±	Pulm. tub. diagnosed 1948. Now
	June 30	—	—	+	+	±	+	+	+	Tub. mammae (PAD). Kahn already for years +. E.S.R. 25.
114		—	—	—	+	—	+	+	—	III-para, children asymptomatic. Kahn during pregnancies —.
115		—	—	—	—	±	—	+	—	II-para, children asymptomatic. Tub. pulm. and Emphysema pulm.. 250 grams of PAS administered.
116		+	—	—	—	—	—	+	—	III-para, children asymptomatic.
117		—	—	±	—	—	—			II-para, children asymptomatic.
118		—	—	+	—	—	—	—		Endometriosis externa f.Dougl. (PAD). Status post salpingo-oophoritidem. E.S.R. 8.
119		—	—	—	±	—	±	+	—	II-para (present. clunium), children asymptomatic. Manual ablation of placenta and alcohol flushing of uterine cavity.
120		—	—	—	—	—	—	+	—	Newborn, clinically asymptomatic, mother I-para and seronegative. Birth weight 3500 grams, weight of placenta 350 grams.
121		—	—	+	—	—	—	—		Newborn, clinically asymptomatic. Mother V-para, all children healthy. Birth weight 3650 grams, weight of placenta 500 grams.
122	June 20	—	—	±	—	—	—	+	—	V-para, Anaemia sec. (Hgb 58
	June 26	—	—	+	—	—	—	+	—	%), children asymptomatic.
123		—	—	—	—	—	—	+	—	I-para, child asymptomatic.
124		—	—	—	±	±	±	+	—	I-para, child asymptomatic.

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
125		—	—	—	—	—	—	±	—	Newborn, clinically asymptomatic. Mother III-para and seronegative. Birth weight 3580 grams, weight of placenta 400 grams.
126		—	—	—	—	—	+	—		Nil.
127		—	—	+	—	—	—			I-para, child asymptomatic.
128		—	—	—	—	—	—	—	±	V-para, all children asymptomatic.
129	July 14	—	—	±	—	—	—	—	—	Otitis media tub.. Pulm. tub..
	July 17	—	—	±	—	—	—	—	—	E.S.R. 80. Pleuritis exsud..
130	July 11	—	—	±	—	—	—	—	—	Sinusitis max. chr. l.s., Ethmoiditis chr. l.s.. E.S.R. 10.
	July 13	—	—	—	—	—	—	—	—	
131		—	—	+	—	—	—			Operation for straightening nasal septum. E.S.R. 8.
132		—	+	—	—	—	—	—	—	Slow extrasystolia, otherwise asymptomatic.
133		—	—	+	—	—	—			Tendovaginitis crepitans.
134	June 26	—	—	±	—	—	—	—	—	Chronic Nephritis. Uraemia.
	July 3	—	—	—	—	—	—	±	—	Bronchitis. E.S.R. 48.
135	Apr. 12	—	—	—	±	+	+	±		Pregnancy, husband seronegative.
	Apr. 30	—	—	+	+	+	+			
	June 13	—	—	+	—	—	—	+	—	
136		—	—	±	—	—	—			Marasmus senilis.
137	June 8	—	—	±	—	—	—			Child aged 13, Pediculosis capitis. 6 th child, sisters and brothers healthy, mother healthy.
	June 12	—	—	±	—	—	—	—	—	
138		—	—	±	—	—	—	—	—	Ephysema pulm.. Chr. Bronchitis. Myodeg. et insuff. cordis.
139		—	—	—	—	—	—	±	—	Ménière's Syndrome. Hysteria?
140	May 30	—	—	—	—	±	—	±	—	Acute infection of unknown origin. Mitral insufficiency.
	July 2	—	—	—	±	—	—	±	—	
141	June 21	—	—	—	—	—	—	±	—	Pulm. tub..
	July 2	—	—	—	±	—	—	±	—	
142	June 7	—	—	—	±	+	±	±	—	May 15 and 22 typhoid-paratyphoid vaccination.
	June 14	—	—	±	—	—	—	±		
143	June 5	—	—	—	±	±	±	±	—	Nil. June 13, Kahn —.
144	June 1	—	—	—	—	—	—	±	—	Acute infection. Sinusitis max..
	June 4	—	—	—	—	—	—	—	—	Myalgia nuchae.
145		—	—	—	—	—	—	±		Spinal tuberculosis.
146		—	—	—	+	—	±	—		Acute eczema.
147		—	—	±	—	—	—			Lumbago.

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
148	May 12	—	—	+	—	±	—			Arthrosis deformans.
	June 28	—	—	+	—	—	—	±	—	
149	Apr. 24	—	—	±	—	—	—			Pregnancy.
	May 21	—	—	±	+	+	+			
	July 2	—	—	+	±	±	—	—		
	July 7	—	—	+	—	—	—	+		
150		—	—	—	—	—	—	+	—	Pregnancy VI month.
151	June 9	—	—	—	±	+	+	±	—	Pregnancy.
	June 21	—	—	—	—	—	—	—	—	
152		—	—	±	+	+	±	—	±	Oedema Quincke.
153	May 10	—	—	+	—	—	—			Nil. Kahn has for long periods
	May 24	—	—	+	—	—	—	+		varied between + and —.
154	May 29	—	—	+	—	—	—	—	—	Pneumonia l.dx.. Anaemia.
	June 2	—	—	+	—	—	—	+	—	
155	Apr. 27	—	—	+	—	—	—			Nil. (Cerebrospinal fluid norm.,
	May 9	—	—	+	—	—	—			thorax Roentgen normal).
	May 16	—	—	+	—	—	—			
	June 5	—	—	+	—	—	—	±	—	
156	April 6	—	—	±	—	—	—	—	—	Pernicious Anaemia. Epidermo-
	April 18	—	—	—	—	—	—	—	—	phytia.
157	May 10	—	—	—	±	+	±			Dystonia ventr..
	May 16	—	—	±	—	—	—			
158	Apr. 25	—	—	±	—	—	—			Acute Appendicitis. Appendic-
	Apr. 29	—	—	—	—	—	—			ectomy.
159	July 18	—	—	—	±	—	±	±	—	Nil. July 27, Kahn —.
160	May 24	—	—	+	—	—	—	+		Pregnancy V month, Hepatitis
	June 2	—	—	—	—	—	—	—	—	5 years ago.
161		—	—	±	—	—	—	—	±	Pregnancy VII month. Delivery 8 months ago, child healthy.
162	Apr. 19	—	—	±	—	—	—	—		Acute nephritis.
	Apr. 26	—	—	+	—	—	—			
163		—	—	—	±	—	±	±	—	Pregnancy III month (3 healthy children).
164		—	—	—	—	—	—	+	—	Pregnancy V month.
165		—	—	—	±	±	—	—	—	Pregnancy V month. Empyema pleurae in 1932.
166	June 16	—	—	—	—	—	—	±	—	Pregnancy IV month.
	June 25	—	—	—	—	—	—	±	—	
167	June 22	—	—	±	—	—	—	±	—	Asthmatic bronchitis. Myodeg.
	June 26	—	—	—	—	—	—	—	—	et insuff. cordis.
168		—	—	—	—	—	—	±	—	Colon irritable. E.S.R. 17.
169		—	—	—	±	±	±	±	—	Tonsillitis recid.. Stenosis ost. atriovent. sin.. E.S.R. 4.

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
170	June 1	—	—	—	±	±	±	—	—	Rheumatoid arthritis now acute.
	June 5	—	—	—	—	—	—	—	—	E.S.R. 38.
171	July 16	—	—	+	—	—	—	—		Cholelithiasis.
	July 19	—	—	—	—	—	—	—	—	
172		—	—	—	—	—	—	±	—	Puerperium (child healthy), Fissura ani.
173		—	—	+	—	—	—		—	Pyosalpinx. Myoma ut.. Inguinal hernia.
174		—	—	—	—	—	—	+	—	Ca vesic. urin.. Hypertonia.
175		—	—	—	—	—	—	+	—	Peritendinitis reg. tarsi.
176		—	—	±	—	—	—	—	—	Amputatio metacarp. No. I m. dx. (under ether narcosis). In 1950 Kahn —.
177	May 31	—	—	—	—	±	±	—	—	Nil.
	June 7	—	—	—	—	—	±	+	—	
178		—	—	—	—	—	—	+	—	Fistula bombisina after appendicectomy. Operation.
179	June 29	—	—	+	—	±	—	±	+	Extensive lacerations to the
	July 3	—	—	+	—	—	—	—	+	finger of left hand. Skin graft.
180	July 6	+	—	±	—	—	—	—	—	Herpes zoster.
	July 11	+	±	—	—	—	—	—	—	
181	July 10	—	—	—	—	—	—	±	—	Intestinal tuberculosis.
	July 11	—	—	—	—	—	—	±	—	
182		—	—	—	—	—	—	±	—	Bronchitis. Insuff. cordis.
183	June 30	—	—	—	+	±	+	+	—	Pregnancy VI month.
	July 17	—	—	—	—	—	—	±		
184		—	—	—	—	—	—	+		Pregnancy III month.
185	May 2	—	—	±	—	—	—	—		Hepatitis. Nephritis. Ulcus corn.
	May 4	—	—	±	—	—	—	—		nae o.dx..
186	July 5	+	+	+	—	—	—	—		Pregnancy VII month. Child-
	August 2	—	—	—	—	—	—	—		birth on Nov. 18, living ♂ child: 3500 grams. Child clinically healthy. Previously 2 healthy children (1942 ♂ 3200 grams, 1944 ♂ 3500 grams). No abortions. No possibility of congenital syphilis. Husband healthy.
187		—	—	—	—	—	—	+	—	Pneumonia l.dx..
188		—	—	+	±	±	±	—		Chronic bronchitis. Pregnancy IV month.
189	Apr. 4	—	—	—	—	—	—		+	Bronchiectasiae et abse. multipl.
	Apr. 6	—	—	—	—	—	—		±	pulm. l.a.. Ca. ventr..

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
190	Apr. 9	—	—	+	+	+	+		+	Pregnancy IX month.
	Apr. 13	—	—	+	+	+	+		+	
	Apr. 24	—	—	+	—	—	—			
	May 16	—	—	—	±	±	±			
	May 23	—	—	+	—	—	—		+	
191		—	—	—	+	+	+		+	Pregnancy.
192		—	—	±	—	—	—	—		Pregnancy VII month.
193		—	—	—	+	±	±	±	—	Asthenia.
194	June 12	—	—	+	—	—	—	+		Blood donor. Inoculation hepatitis.
	June 19	—	—	+	—	—	—	+		
195	Apr. 4	—	—	+	—	—	—	—	—	Pregnancy VIII month. Pernicious anaemia. Death in connection with delivery.
	June 19	—	+	+	—	—	—	+	—	
	June 26	+	+	+	±	—	±	+	—	
	July 7	—	—	+	—	—	—	+	—	
196		—	—	—	—	—	—	—	+	Pregnancy.
197		—	—	+	+	+	+		+	Adenocarcin. ovari l.a.. Carcinosis perit..
198		—	—	—	—	—	—		±	Insuff. cordis.
199		—	—	±	—	—	—			Broncho-pneumonia.
200	May 10	—	—	+	—	—	—			Bronchitis. Caries dentis. Dystonia neuroveget..
	May 15	—	—	+	—	—	—			
201		—	—	—	—	—	±		—	Pregnancy II month.
202	Apr. 14	—	—	±	—	—	—		—	Hypertonia. Pleuritis in 1942 and 1943.
	Apr. 20	—	—	—	—	—	—			
203	Apr. 24	—	—	±	—	—	—			Acute infection. Dyspepsia anacida.
	Apr. 30	—	—	—	—	—	—			
204	Apr. 19	—	—	—	—	—	—		±	Endarteritis oblit.. Incipient gangrene of left foot. Pneumonia in 1943 and Hepatitis in 1946.
	May 4	—	—	—	—	—	—			
205		—	—	—	—	±	±			Eczema idiosyncr..
206		—	—	±	—	—	—			Pterygium oc. sin. post corros. cornae.
207		—	—	±	—	—	—			Pregnancy.
208		—	—	—	—	—	—	+	—	Bronchitis. Pregnancy VI moth.
209	June 29	—	—	—	±	±	±	—		Pleuritis. Childbirth.
	July 7	—	—	—	±	±	±	±	—	
210		—	—	—	+	±	+	±	—	Pregnancy. Asthenia. Anaemia.
211	July 6	—	—	±	—	—	—	—	±	Bronchial asthma.
	July 11	—	—	—	—	—	—	—	±	
212		—	—	±	—	—	—	±	—	Ca ventr.. Haematemesis.
213	Apr. 25	—	—	+	—	—	—			Hydrocele l.dx.
	Apr. 26	—	—	—	—	—	—			

No. of Patient	Date	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II	Remarks
214	June 16	—	—	—	—	—	—	±	—	Pernicious anaemia. Gastric
	June 21	—	—	—	—	—	—	+	—	ulcer (Ca?).
215		—	—	+	—	—	—	—	—	Leuchaemia myeoloica.
216		—	—	—	±	±	—	±	—	Chronic tonsillitis. Cerebrospinal fluid normal.
217		—	—	—	±	—	±	±		Ca corp. ut. (Roentgen treatment). Diabetes mellitus. Myodeg. et insuff. cordis.
218		—	—	—	±	—	±	—	—	Bronchitis. Gastritis. Coliuria.
219	May 24	—	—	—	+	±	±	—		Post-pleurisy condition. Tub.
	May 23	—	—	—	±	±	±	—		lymph.hili. Pregnancy IX month.
220		—	—	—	+	±	—			Nil.

None of the patients presented in Table 23 had been given specific antisyphilitic treatment, and the treating physician concerned did not consider these positive results called for any measures.

In Table 24 are gathered the absolute numbers of the examined false positive results for the reactions employed. Strong and weak reaction results are given in separate columns.

Table 24.

Absolute Numbers for the Reactions Employed of False Positive Results Accounted for

	WR	Chol. WR	Kahn	VDRL	Rein — Bossak	Sitol.	Berger	MCR II
Strongly positive results ..	5	5	77	40	38	32	59	16
Weakly positive results ..	0	1	66	49	53	65	85	21
Total	5	6	143	89	91	97	144	37

Table 25 gives the false positive results accounted for as a percentage of the total explained positive material, by each reaction employed.

It is not likely that a great error is made in assuming that the distribution of false positive results is the same in the unexplained positive material (See Table 16) as in the positive material accoun-

Table 25.

False Positive Results accounted for as a percentage of the Total Number of Explained Positive Results

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Explained positive results	185	310	934	811	811	810	566	372
False positive results included in the above	5	6	143	89	91	97	144	37
Percentage	2.7	1.9	15.3	11.0	11.1	12.0	25.4	10.0

ted for (See Table 25). Table 26 presents the proportion of false positive results of the total material tested. The figures in brackets represent the number of false positive results most probably occurring for each reaction in the unexplained material. This number has been calculated on the basis of the percentages in Table 25 and added to the number of the explained false positive results given in Table 24.

Table 26.

Distribution of False Positive Results between the Reactions Employed in the Total Material Tested

	WR	Chol. WR	Kahn	VDRL	Rein-Bossak	Sitol.	Berger	MCR II
Number of sera investigated	7928	7928	7928	7928	7928	7928	4414	4260
False positive results included in the above	6	7	172	105	108	113	180	43
	(1)	(1)	(29)	(16)	(17)	(16)	(36)	(6)
Percentage	0.08	0.09	2.2	1.3	1.3	1.4	4.1	1.0

In order to show what kind of diagnosis had been made for the patients from whose sera false positive results were obtained, Table 27 has been compiled on the basis of the data in Table 23.

In connection with this Table it must be taken into consideration that the same patient may have been several different diseases all of which could have had an effect. On the other hand, all the reactions presented in Table 23 are not included in this list. When

Table 27.

Incidence of Certain Diagnoses in Patients from Whose Sera False Positive Seroreactions for Syphilis Were Obtained

Disease or Condition	Number of Cases	Disease or Condition	Number of Cases
Bronchitis	9	Hypertonia	5
Pneumonia	8	Malignant neoplasm	9
Bronchial asthma	3	Schizophrenia	6
Pleuritis	3	Manic-depressive psychosis	2
Sinusitis	3	Undefined psychosis	2
Pulmonary emphysema	2	Neurosis	2
Pulmonary tuberculosis	7	Asthenia	3
Other tuberculosis	5	Eczema	4
Salpingo-oophoritis and pyosalpinx	5	Diabetes mellitus	2
Acute infection of unknown origin	5	Struma nodosa toxica and str. adenomatosa foetalis	2
Gastroenteritis and acute enterocolitis	4	Peptic ulcer	2
Appendicitis	3	Leuchaemia	2
Acute hepatitis	3	Prolapsed urethra	2
Coliuria	3	Nephropathiae	8
Helminthiasis	2	Anaemia hypochromica	7
Vaccinations	3	„ hyperchromica	3
Myodeg. et insuff. cordis	11	Pregnancy	44
		Childbirth	15
		Nil	21

comparing the large number of pregnancies with other diagnoses, it must be remembered that they derived from roughly 7000—10000 examined sera of pregnant women. The majority of these sera were investigated with the Kahn reaction only, and where this reaction gave rise to suspicion, other reactions were also made. If all the cases of disease of the lung and upper respiratory tracts, including pulmonary tuberculosis, are combined, there is a total of 35, this number rises to 46 if cardiac insufficiency cases are also included. In these, as is well known, pathological changes or conditions in the lungs are very frequently found. A separate group of the various infections in this list (bronchitis, pneumonia, pleuritis, sinusitis, tuberculosis, salpingo-oophoritis and pyosalpinx, acute infection, gastro-enteritis and entercolitis, appendicitis, hepatitis, coliuria and helminthiasis) would amount to 60 cases.

Table 28 shows the incidence of false positive results among the tested sera of certain special wards. The figures in brackets refer to the number of the patients from whose sera false positive results were obtained.

Table 28.

Proportion of False Positive Results of the Tested Sera of Certain Special Wards with Eight Seroreactions

Special ward or Hospital	Examined Sera	False Positive Results	Percentage
Medical Clinic	928	49 (36)	5.3
Surgical Clinic	972	29 (26)	3.0
Gynecologic-obstetrical Clinic	697	22 (19)	3.2
Otologic, Ophthalmologic and Pediatric Clinic	380	10 (7)	2.6
Asylums	269	12 (10)	4.5

In order to find out what the specificity of our Kahn reaction really was, since it was the only reaction normally used in the routine examination of pregnant women, and to show the frequency incidence of false positive results in the material when only one reaction is used in the examination, the author re-examined the material covering 26,432 sera from pregnant women examined in the Department of Bacteriology and Serology of the University of Turku from January 1, 1950 — October 11, 1951. In this material each serum for which the Kahn was positive or suspected to be positive, was re-examined on the following day using the Kahn, WR and chol. WR. Only after this second Kahn investigation were the sera considered to react positively or negatively. There were 421 Kahn-positive sera in this material, derived from 258 mothers. On each mother whose serum proved to be Kahn-positive a questionnaire was sent to the Maternity Advisory Center concerned. This questionnaire included the following questions:

Has syphilis been diagnosed in the mother concerned and, if so, when?

Has the syphilis been treated and, if so, to what extent?

How many deliveries has the mother had previously (year of birth, birthweight and sex of children)?

How many abortions (and in which month of pregnancy?) has the mother had?

If the delivery has already taken place, please supply the following information about the infant: Birth-weight? sex? result of blood tests? weight of placenta?

How many times were tests taken during this pregnancy and what were the results?

Was the mother treated for syphilis during the pregnancy?

If it is evident that the mother has no syphilis, and that the result of the blood test is false, had the mother at the time of the blood test had any disease or condition that might account for this false reaction (pneumonia, bronchitis, pleurisy, malaria, erysipelas, acute inflammatory disease, pulmonary tuberculosis, cancer, jaundice, recent vaccination, etc.) or is there any possibility of congenital syphilis?

What is your own opinion about the matter and about the family conditions of the mother concerned, is there anything which might indicate possible syphilis?

The required information was received about 142 mothers; 250 Kahn-positive sera in our material were derived from them. With regard to 116 mothers from whom Kahn-positive sera were derived, no information was received. The absolute figures on the average positivity of these unexplained sera for the WR and chol. WR are given in Table 29.

Table 29.

Number of Positive Results Yielded by WR and chol. WR in the unexplained Kahn-positive Pregnancy Material

	WR	Chol. WR	Kahn
Positive	34	61	171
Negative	137	110	—
Total	171	171	171

Out of the 250 explained positive Kahns 183 proved to be specific results, while 67 positive Kahn results must be considered as false. Table 30 gives the WR and chol. WR results for these sera with false positive Kahn results.

Table 30.

Number of Positive Results Yielded by the WR and chol. WR in the Pregnancy Material with False Positive Kahn Results

	WR	Chol. WR	Kahn
Positive	1	3	67
Negative	66	64	—
Total	67	67	67

The proportion of false positive Kahn results of the total explained material of the Kahn-positive sera (250) of pregnant women is 26.9 %. On the assumption that false positive results are similarly distributed in unexplained and explained cases, the probable total of the false positive Kahn results in the whole treated material has been assessed at 113. Table 31 shows the proportion of false positive results of all the tested sera of pregnant women where only one reaction — the Kahn — was used.

Table 31.

Incidence of False Positive Results in the Sera of Pregnant women Tested with the Kahn Reaction alone

Tested Sera	False Positive Kahn Results	Percentage
26,432	113	0.43

In reality the material of the sera of pregnant women tested with the Kahn reaction revealed a lower percentage of false positive results than the present original material of 7,928 sera, chiefly comprising diseased people. A comparison of Tables 28 and 31 shows the number of the sero-reactions used in the examination affects the incidence of false positive results in the material involved.

The Cardiolipin and Sitolipin Antigen in the Complement Fixation Reaction

Material and its treatment

The material comprises 5,116 sera, which were all tested by the routine reactions employed — the WR, chol. WR and Kahn — and

by the Wassermann reactions performed with cardiolipin and sitolipin as the antigens (card. WR and sitol. WR). Using these four complement fixation reactions 238 cerebrospinal fluid samples were also tested.

The serum samples were derived from the following sources:

Medical Clinic of the University of Turku	877	samples
Surgical Clinic „ „ „ „ „	1,019	„
Gynecologico-Obstetrical Clinic „ „	790	„
Dermato-Venerological „ „ „	154	„
Pediatric, Oto-Rhino-Laryngological and Ophthamological Clinic of the University of Turku	467	„
Pori General Hospital	467	„
Asylums (Turku, Halikko, Harjavalta, Uusikaupunki, Seili, Asylum for Prisoners)	285	„
Other Hospitals	276	„
Venereal Diseases Outpatients Clinics in Turku and Pori	173	„
General Practitioners	556	„
Advisory Centers	122	„
Prisons	55	„
Factories, Blood Service, Poor Law Institutions, etc.	106	„
Total	5,116	samples

As this test series was performed immediately after the investigation into the flocculation reactions it was no longer possible to obtain equally detailed information about the positive cases. Thus, no efforts were made to check the specificity of the positive results obtained for the Kahn, but it was considered sufficient to find out whether syphilis was involved or not in the cases for which the complement fixation reactions yielded positive results.

Average Sensitivity of the WR Carried Out with Four Different Antigens and Kahn Employed

When the material of 5,116 sera was tested by the WR carried out with four different antigens and Kahn, 593 positively reacting sera were found. Table 32 shows the average sensitivity of each of the reactions.

Table 32.

Average Sensitivity of the WR Carried Out with Four Different Antigens and Kahn in the Positive Serum Material

	WR	Chol. WR	Card. WR	Sitol. WR	Kahn
Positive	163	253	338	324	566
Positivity %	27.5	42.7	57.0	54.6	95.4
Negative	430	340	255	269	27
Total	593	593	593	593	593

As can be seen from the Table, the seropositivity with these reactions, bears a certain correlation to the sensitivity of the Kahn. Table 33 shows the behaviour of the WR's carried out with different antigens in the 27 cases for which the Kahn was negative.

Table 33.

Behaviour of the WR's Performed with Four Different Antigens in Cases in which the Kahn Was Negative

	WR	Chol. WR	Card. WR	Sitol. WR
Positive	10	8	16	16
Negative	17	19	11	11
Total	27	27	27	27

Table 34 shows the frequency with which each of the reactions employed occurred as the only positive reaction.

Table 34.

Occurrence of the WR Carried Out with Four Different Antigens and Kahn as the Only Positive Reaction, in Absolute Figures

	WR	Chol. WR	Card. WR	Sitol. WR	Kahn
Number of samples	3	1	4	2	210

It should also be mentioned that in 7 cases the card. WR and sitol. WR together were positive while the other reactions employed were negative. The combination WR —, chol. WR —, card. WR +,

sitol. WR +, Kahn + appeared in the material 77 times. The card. WR and sitol. WR gave different results for 37 samples (0.7 %) and similar results for 5,079 samples (99.3 %).

Results of the WR Carried Out Quantitatively with Four Different Antigens

The WR was performed quantitatively with all the four antigens on 22 samples only. Since the material was so limited the titration results for all the 22 samples is listed below. The last positive tube has been taken as the limit of titration. A geometric dilution series was used, the dilution being: whole serum, 1 : 2, 1 : 4, 1 : 8 etc. corresponding to the final dilutions 1 : 5, 1 : 10, 1 : 20, 1 : 40 etc. The degree of dilution is not indicated in the list of cases. In the cases investigated the following results were obtained:

Case 1.	WR +	Case 2.	WR —
	chol. WR +++		chol. WR —
Treated	card. WR +++	Treated	card. WR ++
Syphilis	sitol. WR ++±	Syphilis	sitol. WR +
Case 3.	WR —	Case 4.	WR —
	chol. WR —	Syphilis	chol. WR +
Treated	card. WR +++		card. WR ++
Syphilis	sitol. WR +		sitol. WR +
Case 5.	WR ++	Case 6.	WR —
Syphilis	chol. WR ++		chol. WR +
	card. WR ++++	Treated	card. WR ++
	sitol. WR +++	Syphilis	sitol. WR ++
Case 7.	WR —	Case 8.	WR +
	chol. WR —	Syphilis	chol. WR +
Treated	card. WR ++		card. WR ++++
Syphilis	sitol. WR +		sitol. WR +++
Case 9.	WR ++	Case 10.	WR +++
	chol. WR +++	Syphilis	chol. WR +++
Treated	card. WR +++		card. WR ++++++
Syphilis	sitol. WR +++		sitol. WR ++++

Case 11.	WR	—	Case 12.	WR	++++
Aortitis	chol. WR	±	Syphilis	chol. WR	+++
syphilitica	card. WR	++		card. WR	+++±±
	sitol. WR	++		sitol. WR	+++
Case 13.	WR	++	Case 14.	WR	++++
	chol. WR	+++	Untreated	chol. WR	++++
Treated	card. WR	++++	latent	card. WR	+++++
Syphilis	sitol. WR	+++	Syphilis (Nov. 30)	sitol. WR	+++++
Case 14. Control	WR	++++	Case 15.	WR	+++
Sample	chol. WR	++++	Syphilis	chol. WR	+++
(Dec. 2)	card WR	+++++		card. WR	++++
	sitol. WR	+++++		sitol. WR	++++
Case 16.	WR	—	Case 17.	WR	++
	chol. WR	+	Syphilis	chol. WR	+
Treated	card. WR	+++		card. WR	++
Syphilis	sitol. WR	++		sitol. WR	+
Case 18.	WR	+	Case 19.	WR	—
Treated	chol. WR	++++	Syphilis	chol. WR	—
Congenital	card. WR	++++++++		card. WR	++
Syphilis	sitol. WR	++++++++		sitol. WR	++
Case 20.	WR	+	Case 21.	WR	+++++
	chol. WR	+	Pneumonia	chol. WR	++++++
Treated	card. WR	+++		card. WR	+++++
Syphilis	sitol. WR	++		sitol. WR	+++++

In cases where syphilis only is given as diagnosis, the stage of the disease and the possible treatment were not known. When examining the results, we find that in all syphilis cases of the present material the card. WR represents the most advanced positivity in the dilution series. The sitol. WR was positive together with the card. WR for 21 syphilitic sera in the same dilution stage in 9 samples, remained one dilution stage behind in 9 samples and two dilution stages behind in 3 samples. The chol. WR positivity in these 21 syphilitic sera extended to the same dilution stage as the card. WR in two samples, remained one dilution stage behind in 8, two dilution stages behind in 4 and three dilution stages behind

in 3 samples; chol. WR was completely negative in 4 samples. The WR-positivity of the same sera extended to the same dilution stage as the card. WR in 1 sample, remained one dilution stage behind in 5, two dilution stages behind in 4, three dilution stages behind in 2 and four dilution stages behind in 1 sample; the WR was completely negative in 8 samples. In case No. 21, considered as false positive, which will be commented on later, the chol. WR-positivity extended farthest in the dilution series, the WR, card. WR and sitol. WR remaining one dilution stage behind it.

False and Doubtful Positive Results Obtained for the WR Carried Out with Four Different Antigens

False or doubtful positive complement fixation reactions were obtained for sera of 12 patients. Table 35 gives case-list of these patients. They all denied a history of syphilis.

Table 35.

Case-List of Patients from Whose Sera False or Doubtful Positive Complement Fixation Reactions Were Obtained

Patient	Date *	Serologic status					Remarks
		WR	Chol. WR	Card. WR	Sitol. WR	Kahn	
1. M. Child	Oct. 5	—	—	—	—	±	Newborn. *Mother:* 36 yrs. Engineer's wife. Married at 17. Previously 3 children (— 34: 2500 g, — 38: 3500 g, — 41: 3200 g) all living and healthy. In 1949 angina, after which continuous pains in joints. No abortions. During present pregnancy continuous nausea, recently in bed for 2 weeks for this reason. Has taken during this pregnancy 200 tablets of Ca and 100 tablets of Fe. At the beginning of delivery Hgb 67 %, alb. —. The child was born in second occiput presentation, delivery normal. Child's
	Oct. 9	—	—	+	+	±	

* Date has been given only when control examinations have been made.

Patient	Date	Serologic status					Remarks
		WR	Chol. WR	Card. WR	Sitol. WR	Kahn	
							birthweight 3430 g., weight of placenta 450 g. Child clinically healthy. Mother's blood test on Oct. 5 ———— ±, on Oct. 6 ————. *Evaluation:* The clinicians have considered the results false; due to the increasing seropositivity the present author would, however, consider the case doubtful.
2. R.L.	Nov.	1	—	+	—	—	+	34 yrs., labourer (case 21 in quantitative investigations) single. In Nov. — 49 in hospital with dgn.: Pleuritis exs.l. sin., WR —, chol. WR —, Kahn —. Now fever about 1 week and cough. Thorax rtg: In lower part of upper lobe of left lung scattered pneumonic infiltrate over a handbreadth area. E.S.R. 30 mm/1 h. Cerebrospinal fluid: 0. During Oct. 30 — Nov. 9 3.3 million I.U. of penicillin were given and the shading in the lungs became clearer. *Evaluation:* The decrease in seropositivity after the cure of pneumonia takes place so quickly that the present author would consider the results false positive.
	Nov.	6	+	+	+	+	+	
	Nov.	13	+	+	+	+	+	
	Nov.	30	—	+	—	—	+	
	Nec.	13	—	—	—	—	±	
3. N.M.	Nov.	7	—	—	+	—	+	61 yrs., labourer, female, single. No children or abortions. Admitted to hospital on account of tumor of the breast which was operated, pathologico-anat. dg.: Adenocarcinoma scirrhosum mammae. E.S.R. 7 mm/1 h. *Evaluation:* Syphilis dg. not supported by medical history or condition, false positive result.
	Nov.	10	—	—	+	—	+	
4. L.K.	Nov.	8	—	+	—	—	+	39 yrs. shop assistant (female),

Patient	Date	Serologic status					Remarks
		WR	Chol. WR	Card. WR	Sitol. WR	Kahn	
	Nov. 10	—	+	—	—	+	single, fourth of 4 children. Admitted to hospital on account of cough and fever. Thorax rtg.: In lower part of upper lobe of left lung pneumonic shading. AST 80, cold agglutination test 1: 16. Cerebrospinal fluid: Pandy +, Nonne —, sugar 0.065 %, protein 0.02 %, chlorides 0.33 %, WR —, chol. WR —. Antibiotics were administered and the shading in the lungs disappeared and E.S.R. decreased from 34 to 9. Dg.: Acute pneumonia. *Evaluation:* These results appear most probably false positive.
	Nov. 15	+	+	—	—	+	
	Nov. 20	±	+	—	—	+	
5. H.A.	Nov. 13	—	—	—	±	—	29 yrs., policeman's wife. IV-para. Earlier deliveries: — 43: 1750 g, — 45: 3500 g, — 48: 3670 g. All children living and healthy. No abortions. Hgb. 69 %. Child: 3600 g. clinically and serologically healthy. Placenta 550 g. Delivery normal. *Evaluation:* False positive result.
6. K.M.	Nov. 26	—	+	—	—	±	16 yrs. Girl, third of 4 children sisters and brothers anamnestically healthy. For 4 days fever and sharp pains when breathing. Dg.: Pleuritis exs.l.sin., Congenital weakness of the heart (defectus septi interventricularis?) E.S.R. when admitted to hospital 51 mm/1 h., after 2 weeks 23 mm/1 h. *Evaluation:* False positive results.
	Nov. 28	—	—	—	—	—	
7. K. Child	Nov. 29	+	+	—	—	—	Newborn. Mother: 33 yrs. driver's wife, married for 12½ years. In 1948 appendicectomy, otherwise been healthy Earlier deliveries: — 40: 2800 g, living, — 42: 1500
	Nov. 30	—	—	—	—	—	

Patient	Date	Serologic status					Remarks
		WR	Chol. WR	Card. WR	Sitol. WR	Kahn	
							g premature stillbirth, — 43: 3800 g living. Both older living chlidren healthy. No abortions. During pregnancy mother took 500 tablets of Ca and 200 tablets of Fe. Mother's blood tested twice during delivery, all reactions negative. Delivery normal, child clinically healthy, weight 3740 g, weight of placenta 600 g. *Evaluation:* False positive results.
8. M.E.	Nov. 30	—	+	—	—	+	43 yrs., labourer, married, 2 healthy children. Fever and cough for 3 weeks. X-ray and clinical dg.: Pneumonia l.sin., Tub. pulm. l.a. E.S.R. on admission to hospital 65 mm/1 h., on discharge 58 mm/1 h. Leucocytes, on admission 15,700. TB —. *Evaluation:* False positive results.
	Dec. 4	—	—	—	—	+	
9. S.H.	March 19	+	+	—	—	+	40 yrs. factory worker third of 3 children. Married, 5 healthy children. For 2 — 3 weeks fever and cough, expectorations bloody. Axial temp. when admitted 40° C. Thorax rtg.: Scattered shading in both lungs as in bronchopneumonia: after 1 week the shading had decreased noticeably. Cerebrospinal fluid: 0. TB —. E.S.R. on admission 110/mm/1 h. on March 13, the result of serologic examination was WR ±, chol. WR +, Kahn +. *Evaluation:* False positive results.
10. H.E.	Oct. 20	—	+	—	+	—	Man (age and occupation unknown). On Oct. 12 typhoid vaccination. In the same evening axial temp. 30° C. Took 24 tablets of sulpha on Oct. 13 — 14. Fever persisted, cough and pains

Patient	Date	Serologic status					Remarks
		WR	Chol. WR	Card. WR	Sitol. WR	Kahn	
							in throat — had repeated sore throats. Admitted to Pori General Hospital Oct. 17. with slight erythema on the skin. Was given 6 × 300,000 I.U. of penicillin and was discharged healthy. Nothing indicative of syphilis was observed in the condition. Dg.: Acute infection. *Evaluation:* Probably false positive results.
11. E.P.	Oct. 22	—	—	+	—	+	Man (age and occupation unknown). Treated in an asylum for schizophrenia. In 1945 and 1950 WR —, chol. WR —, Kahn —. On April 16 Tub. pulm. l.sin. was diagnosed, which by the time of blood sample collection had somewhat improved. *Evaluation:* False positive results.
12. L.V.	Nov. 29	—	—	+	+	+	Specimen from antenatal advisory center. 5th pregnancy. Earlier deliveries: — 45: 4500 g, — 47: 3700 g, — 48: 3500 g, — 49: 3700 g. All children living and healthy. No abortions. Earlier always healthy. Progress of pregnancy normal. On Aug. 1 and Aug 8 Kahn had been +. Pregnancy terminated on Feb. 11 in normal delivery, child's weight 3500 g, clinically healthy. Blood test data unknown. *Evaluation:* Probably false positive results.

If we reck on all these cases presented in Table 35 to be false positively reacting, as in fact has been done clinically, the false positive results occur in each WR performed with different antigen in the frequency shown in the Table 36.

Table 36.

Distribution of False Positive Results between the WR's Performed with Different Antigens and Their Percentages of the Total Investigated Material

	WR	Chol.WR	Card.WR	Sitol.WR
Investigated material	5,116	5,116	5,116	5,116
False positive results	6	13	7	6
Percentage	0.12	0.25	0.14	0.12

From table 35 it can be seen that 19 out of 24 samples collected from these 12 patients proved to be Kahn-positive.

Table 37 tabulates the diagnoses of the 12 cases described in Table 35.

Table 37.

Diagnoses of the 12 Patients from whose Sera False Positive Results Were Obtained with the Complement Fixation Reactions

Disease or Condition	Number of Diagnoses
Pneumonia	4
Pleuritis	1
Pulmonary tuberculosis	2
Acute infection of unknown origin	1
Typhoid Vaccination	1
Malignant neoplasm	1
Healthy newborn infant	2
Childbirth	1
Pregnancy	1
Schizophrenia	1

Of special interest in the author's opinion is the following case in which the liquor, obtained by knee-puncture, was examined with the WR using these four different antigens and Kahn.

Convict, aged 34, denied history of syphilis. For a week he had had a temperature rising to 40.1° C and cough, with swelling at the knee-joint. Hospital diagnosis: Pleuropneumonia l.sin., Gonitis l.dx. E.S.R. was 94 mm/1 h. The knee was punctured and the fluid obtained was investigated syphilis-serologically, like a serum. The result was: WR +, chol. WR +, card. WR —, sitol. WR —, Kahn —. In the serum of the same patient all these 5 reactions were negative.

This was clearly a case where a septic condition was concerned, occurring simultaneously with pneumonia and gonitis. The WR and chol. WR obtained from the knee fluid were probably false positive while the card. WR, sitol. WR and Kahn were negative.

Results of Cerebrospinal Fluid Samples Tested with the WR Carried out with Four Different Antigens

Using the WR, chol. WR, card. WR and sitol. WR 238 cerebrospinal fluid samples were investigated. Positive results were obtained from 25 samples derived from 21 patients. The distribution of positive results between the WR's performed with different antigens is presented in Table 38.

Table 38.

Numerical Distribution of Positive Results between WR's Carried out with Different Antigens in the Cerebrospinal Fluid Specimens Tested

	WR	Chol. WR	Card. WR	Sitol. WR
Number of positive results	23	23	24	24

All the positively reacting samples were derived from syphilities. Thus no false positive results appeared in the material investigated. Different results for the WR's performed with these four antigens were obtained on three samples only. In one of these the card. WR was positive while the three other antigens gave negative results, in the second sample the card. WR was negative while the others gave positive results, in the third sample the card. WR and sitol. WR were positive, but the WR and chol. WR negative.

DISCUSSION OF SEROLOGIC RESULTS

On the basis of the serologic material tested with the WR, chol. WR and Kahn for the years 1946 — 50 it can be considered evident that in each case the average sensitivity of the WR and chol. WR employed is decisively dependent on the proportion the active syphilis represents in the investigated syphilis material at the time of

investigation. Whenever fresh untreated or slightly treated syphilis cases are plentiful, i.e. when the research is done during so called syphilis epidemics, the average percentage of sensitivity of the WR and chol. WR in syphilis cases is high. Thus in 1947, when there was still much fresh syphilis in Finland, there were 67.1 % WR-positive and 87.0 % chol. WR-positive cases. With the decrease of fresh syphilis, the syphilis material to be tested will chiefly comprise old, treated syphilis cases, and then the percentage of the WR and chol. WR-positive cases will go down. In 1950, when fresh syphilis in Finland had already decreased considerably, there were no more than 32.4 % WR-positive and 53.7 % chol. WR-positive cases. In the course of all these years the Kahn-positivity remained largely unchanged at 92 — 97 %. Accordingly, when employing the combination — WR, chol. WR, Kahn — the sector of seropositivity in the investigated material was almost wholly determined by the Kahn-positivity. These results also seem to indicate that the sero-reactions used in the routine examinations should be selected to correspond with the syphilis situation prevailing on each separate occasion. If, at a time when there is little syphilis and the greater part of the possible syphilis material will be old, treated syphilis cases, serologic assistance is still desired in syphilis diagnostics, then the main stress in serological examination should be on the flocculation reactions, especially as the Wassermann reaction is not able to compete with the flocculation reaction in sensitivity, even if effected with the cardiolipin or sitolipin antigens. The most natural routine reaction combination would then seem to be one complement fixation reaction as specific and sensitive as possible and two flocculation reactions. By this means a sector of seropositivity as extensive as possible would be obtained, where this seems desirable, and in addition, the complement fixation reaction used would contribute to the results because of its specificity.

Although in the material covering the years 1946 — 50 there were considerable differences in the sensitivities of the WR, chol. WR and Kahn (Table 1), the percentages of the weak reactions of the positive results were very much the same in range, from 7 % to 10 % (Table 4). In my opinion this seems to indicate that the margin of weak positive cases does not depend so much on the reaction technique and the antigen used, but remains within the same range in various situations.

It is interesting to compare the percentages of the false positive reactions to the WR, chol. WR and Kahn (Table 5) obtained on the basis of the material for the years 1946 — 50, with those which were obtained in the material of 7,928 sera examined in 1951 to illustrate the percentages of false positive results of the whole material tested (Table 26). The former figures were arrived at, as explained above in greater detail, chiefly from the diagnoses given on the sample delivery notes, by observing the results from control samples, by the use of the journals of the clinics of the University of Turku and General Hospital of Pori, and by utilization of the concept "syphilis suspect". Such a method must often be resorted to by force of circumstances when interpreting serologic results. The latter percentage figures were obtained by an attempt to check the specificity of each positive result by applying directly to the physician who sent the sample and by requesting him to give information about the case concerned. Although one must take into consideration in the comparison that Table 5 includes false positively reacting cases on the basis of the result of only one blood sample, and the percentage figures in Table 26 illustrate the proportion of all positive reaction results obtained from the material studied, the differences must nevertheless be regarded as large. Some of the differences may perhaps be explained by the fact that the majority of the Kahn-material for 1946 — 50 at least consisted of advisory center and institution material, while the hospital material proper is smaller; the later material of 7,928 sera, however, is chiefly composed of hospital patients. Of the hospital samples a higher percentage evidently are false positively reacting than the samples from advisory centers (see Tables 28 and 31). Some of this difference is certainly explained also by the fact that the examinations were made at different times and by different persons. Thus some only of the Kahn results for the years 1946 — 50 have been read microscopically, while all the Kahn results of the latter material to be used in comparison were read with a 6 × magnification. Even allowing for all factors of this nature it is evident that the investigation method used for the material covering the years 1946 — 50 treats the results too mildly. Many positive results are erroneously described as specific. It would seem that the method used in dealing with the later material — the immediate check of each positive result by procuring information from the physician delivering the

sample — is more closely related to the truth. It would be of the greatest importance for clinicians to remember that so far no certain means exist by which a laboratory can distinguish a specific result from a false positive result, but that the evaluation of the specificity of the result in the first place is to be made by the clinician who has practical facilities for this.

The present observations concerning the position of the cardiolipin and sitolipin antigens as the antigens in the flocculation reaction are based on the material of 7,928 sera. All these were tested with 6 different reactions, more than 4,000 of these sera were tested with 7 reactions and 3,014 of these sera were tested with 8 different reactions. The purpose of the simultaneous use of so many reactions was to make the sector of seropositivity so extensive that as few syphilis cases as possible would remain outside it. The method appears to have succeeded to a large extent in this respect, because the total of the samples yielding positive results — 1,508 samples giving positive results out of the series of 7,928 consecutive, unselected specimens — is of such a size that, considering the syphilis situation in Finland at present, it most probably also covers the majority of the old and treated syphilis cases included in the material tested. On the other hand, this simultaneous use of many reactions naturally meant that the group of false positive cases swelled considerably (Table 23). The significance of the flocculation reactions as the extenders of this sector of seropositivity can perhaps be seen from the fact that in the investigation series concerning the antigens of the complement fixation reaction, effected immediately after the investigation series concerning the flocculation reactions, the total of positive results rose to 593 samples yielding positive results out of the total number of 5,116 sera.

If on the basis of these investigation series concerning the flocculation reactions and the complement fixation reactions comparisons are made between the cardiolipin and sitolipin antigens, the attention will be attracted throughout by the similarity of the results obtained with these antigens. When used as flocculation antigens no significant differences appear in the percentages illustrating the average sensitivity of the cardiolipin and sitolipin antigens in the different groups of syphilis cases (Tables 11 — 14). In the groups of patients who are at different stages of antisyphilitic treatment, the percentages of the positive results obtained

with the cardiolipin and sitolipin antigens are statistically equally large (Tables 17 — 21), and the differences between the cardiolipin and sitolipin antigens appearing in the curves illustrating the results obtained with quantitatively made flocculation reactions must be regarded as of no practical importance (Fig. 2). Equal numbers of false positive flocculation reaction results were obtained with cardiolipin and sitolipin (Table 26). When the cardiolipin and sitolipin antigens were used in the complement fixation reaction (WR), an equal amount of positive results statistically estimated were obtained for both antigens; the same applies to the false positive results (Tables 32 and 36). In the quantitative complement fixation reaction, however, the postitivity obtained with the sitolipin antigen often tended to remain one or two dilution stages behind the result obtained with the cardiolipin antigen. From Tables 10 and 34 we can see that the cardiolipin and sitolipin antigens rarely appear as the only antigens yielding a positive result. On the other hand they most often appear together as giving positive results. Thus in the investigation series concerning the antigens of the complement fixation reaction, the card. WR and sitol. WR yielded similar results in 99.3 % and differing results in 0.7 % of the cases.

On the strength of these results the author is inclined to regard the cardiolipin and sitolipin antigens as of equal value both in sensitivity and specificity. Their serologic relationship is at least as close as the similarity of their chemical structure.

If we compare the results obtained for the Kahn reaction and the cardiolipin and sitolipin slide tests, on the basis of the results of the investigation series comprising 7,928 sera tested first for the flocculation reactions, we observe that the percentages illustrating the sensitivity of the Kahn reaction are higher throughout than those illustrating the sensitivities of the cardiolipin and sitolipin slide tests. No very large differences are involved, and in the tertiary syphilis group (Table 12), in the groups of untreated latent syphilis patients (Table 17) as well as in the groups of treated latent syphilis cases (group B, Table 19), latent syphilis patients treated chiefly with penicillin (Table 20), and latent syphilis cases treated chiefly with combined Bi-neosalvarsan treatment (Table 21) these differences are not statistically certain. False positive results obtained were clearly more numerous for the

Kahn reaction than for the cardiolipin and sitolipin slide tests (Table 26).

When looking for possible exterior factors which might partially account for the difference in sensitivity between the Kahn reaction and the cardiolipin and sitolipin slide tests, it should first be mentioned that the Kahn reaction results throughout the whole investigation series were read with 6 × magnification. Thus the number of positive results increases, and more false positive results, naturally, are obtained. On the other hand, the number of positive results for the cardiolipin and sitolipin slide tests might perhaps have been increased by using a higher magnification than 6 × in the reading of all reaction results; the false positive results obtained would doubtless have been more numerous also. The use of a loupe for a large material, however, is so much quicker and more advantageous than the use of the microscope that it was not considered advisable to go over to the use of the microscope to achieve the increase in positive results possibly obtainable in this manner. The author's experience was that he could read the same positive results with a loupe as with a microscope. However, no scientifically controlled material can be presented to prove this. Regarding the sitolipin slide test, it must also be remembered that Uroma and Tuomioja (1951 *a*) later altered the percentage composition of the sitolipin antigen used in the slide test, which may contribute towards increasing the sensitivity of the sitolipin slide test. However, the author has not seen any material concerning this matter published. As in the present material the results obtained for the sitolipin slide test broadly speaking conform with those for the cardiolipin slide test, a result in agreement with the results appearing in the literature and the results reported earlier, it is hardly probable that this alteration in the antigen composition would have increased the sensitivity of the sitolipin slide test very much. It must be borne in mind that the comparison is between a tube test and a slide test. The investigation by Uroma and Tommila (1951) consisting of a comparison between the Kahn and the sitolipin slide test has already been mentioned. The total material covered 9,830 samples, out of which 151 yielded positive results for the Kahn and 164 for the sitolipin slide test; the difference must be considered as insignificant. The specificity of the differing cases only was explained;

there were 18 false positive results for the Kahn and 4 for the sitolipin slide test. In the present materials, in the material comprising pregnant sera the Kahn yielded false positive results in 0.43 % of the cases and in the material chiefly derived from hospital patients in 2.2 % of the cases. Accordingly, had we investigated the comprehensive material of UROMA and TOMMILA, covering samples from different hospitals and outpatient clinics, using our own Kahn reaction, the number of false positive results alone would have amounted to 40 — 200 sera, all so-called technical errors included, in this material of nearly 10,000 sera. This calculation is an attempt to show that results obtained in different places for the same reaction are not immediately comparable with each other.

The comparison of the Kahn reaction with the cardiolipin and sitolipin slide tests performed on the basis of the present material could be summarized as follows: in definite syphilis cases the Kahn reaction yields as many as or slightly more positive results than the cardiolipin and sitolipin slide tests. On the other hand, the Kahn reaction gives a higher proportion of false positive results than the two slide tests. If we compare this result with the results reported from the literature in the beginning it is found that the result represents a kind of mean value of these investigations.

Meinicke's second clarification reaction was made on a total of 4,260 samples. The relationship of this reaction to the other flocculation reactions can most clearly be seen from Tables 14 and 26, in which the results obtained on the whole syphilis material, and the false positive results obtained in the whole investigated serum material are compiled. From them we see that in the syphilis cases on the average Meinicke's second clarification reaction yielded a slightly higher number of positive results than the cardiolipin and sitolipin slide tests, the difference, however, being so small as to be statistically insignificant. False positive results obtained again were, on the average, slightly less numerous for Meinicke's second clarification reaction than for the cardiolipin and sitolipin slide tests; however, even this difference is not statistically significant. Compared with the Kahn reaction Meinicke's second clarification reaction yielded in syphilis cases a slightly lower number of positive cases on the average — the difference again is not statistically significant — on the other hand it gave considerably less false positive results.

The results obtained for Meinicke's second clarification reaction are thus fully comparable with the results obtained for the cardiolipin and sitolipin slide tests.

The Berger slide test made according to the original method yielded, taking all the cases into consideration, on an average slightly more positive results than the Kahn reaction proper — this difference was so small that it is not statistically significant (Table 9). From Tables 14 and 26 we can see, however, that this is not true of the syphilis cases, in which the Berger slide test yielded, if the absolute percentages are studied, an even smaller number of positive cases on the average than the Kahn reaction; this difference, however, is not fully significant. On the other hand the Berger slide test yielded disproportionally many false positive results, considerably more than the Kahn reaction (Table 26). When examining the quantitative results, it is found that the Berger positivity does not extend on the average any farther in the dilution series than Kahn positivity (Fig. 3).

On the basis of these results the author is inclined to consider the Berger slide test performed according to the original method — a modification of the Kahn reaction — to be an unsuccessful modification.

In the Berger slide test a 4 minutes rotation was employed. It has been mentioned earlier that FODDEN and MADDOX (1947) recommend a 2 minute rotation, because more prolonged rotation, according to them, might lead to misleading granularity. However, as they did not present any material in support of their observation, the author chose the original method. It may be that a shortening of the rotation time would have decreased the number of false positive results. This is supported by the fact that in MAGNUSSON's (1950) material also referred to previously, the Berger slide test with 2 minutes rotation yielded as many positive results for syphilitic sera as in the present material, while in his material there were as many false positive results for the Berger slide test as for the Kahn reaction.

In the investigation series concerning flocculation reactions the routine reactions also included two complement fixation reactions, the WR and chol. WR. Observing the average percentages of the positive results yielded by these in the groups of patients who had received antisyphilitic treatment of various degrees, the

attention is attracted by the same factor as in the material for 1946 — 50. This showed, it will be remembered, that with the decrease of fresh syphilis the WR and chol. WR positivity percentages of the syphilis cases also decreased. In this material, it is seen that antisyphilitic treatment of different degrees primarily affected the average percentages illustrating the WR and chol. WR positivity, whilst the average positivity percentages concerning the flocculation reactions showed fairly small changes only (Tables 17 — 21).

It is interesting, although a slight digression from our subject, to compare the serologic results in the group of patients treated chiefly with penicillin (Table 20) and in the group of patients treated chiefly with combined Bi + neosalvarsan treatment (Table 21). It is found that in the latter group the percentages illustrating the WR and chol. WR positivity are clearly lower than in the group of patients treated chiefly with penicillin. The differences are statistically significant. In connection with the flocculation reactions there exists a similar difference which, however, is not large enough to be statistically significant. When evaluating the differences, it must be borne in mind that in the group of patients treated chiefly with penicillin the follow-up period will be shorter on an average than in the group of patients chiefly treated with combined treatment, because penicillin treatment on a larger scale has been given in Finland only since 1949 — 50. However, reference is made to PERDRUP's (1951) study as an example. In his material, which comprised 134 patients treated with combined treatment and an equal number of patients treated with penicillin, the seroreactions in the cases in which the titers of the seroreactions were high in the beginning of the treatment, turned negative more slowly in the group of the patients treated with penicillin than in the group of patients treated with combined treatment. LAPIERE and OLIVIER (1952) reported, however, that they had come to the opposite result. As an example of how quickly the seroreactions turn negative in those given combined treatment reference is made to MØLLER's study (1951). His material covered 625 patients to whom combined treatment had been given and who were examined with the Wassermann, Kahn and Meinicke reactions. Of these, 463 patients were in sero-positive stage in the beginning of the treatment. 6 months after the begin-

ning of the treatment 75 % of the patients had become seronegative and in course of one year 91 % of them were seronegative.

When examining the diagnoses appearing in our material in patients from whom false positive seroreactions for syphilis were obtained, it can be seen that the majority are various infections, especially various infections of the respiratory tract (Tables 6, 27 and 37). This agrees with the experiences reported in the literature reviewed previously. When discussing the incidence of false positive results yielded by the Kahn reaction used in the material covering sera of pregnant women, a percentage of 0.43 was arrived at on the basis of a material of 26, 432 speciments, technical errors not then being specified as a separate group. This percentage is the same as was obtained by PENTTINEN (1946) in his doctorial thesis. PENTTINEN came to this result on the basis of card index comparisons and follow-up examinations. In the present material the study of the material was based on information collected by questionnaires from the maternity centers concerned. Both methods, then, led to the same result.

In contrast to the results gained from serum investigations, in the cerebrospinal fluid material the Wassermann reaction yielded equally many positive results independently of whether it was performed by employing beef heart antigen not sensitized with cholesterol, beef heart antigen to which 0.15 % of cholesterol was added, cardiolipin antigen or sitolipin antigen (Table 38).

IV.

Syphilis frequency in Turku and Pori county

SYPHILIS FREQUENCY IN FOREIGN COUNTRIES

In our neighbouring country, Sweden, the average frequency of reported syphilis cases in 1949 was 10.0/100,000. By provinces the highest frequency was 18.6/100,000 and the lowest 1.7/100,000. Of the towns in Sweden, Karlskrona was leading with a frequency of 55.2/100,000, Stockholm came next with the frequency of 21.8/100,000. In Sweden syphilis is most generally found in the parts on the south and east coasts, in the countryside the syphilis frequency varies from 0.6 to 8.3/100,000 (WINTZELL 1950). During the years 1919 — 48 a total of 35,000 syphilis cases were reported in Sweden. According to calculations 22,850 of the patients are living. If all persons over the age of 18 years are taken into consideration in the calculations, the syphilis frequency in Sweden will be 4.3 $^0/_{00}$. In the Swedish maternity advisory centers the syphilis frequency is 5 $^0/_{00}$. In a material covering 17,510 seamen the figure illustrating the syphilis frequency was 20 $^0/_{00}$ (TOTTIE 1950).

IDSØE and VOGELSANG (1950) analysed a material comprising 4,961 pregnant women, examined serologically in Bergen for possible syphilis. 44 syphilis cases were detected, and the syphilis frequency was thus 8.9/1000. In 1947 2.1 % of the soldiers serving in Norway had syphilis; in a brigade staying in Germany for 7 months the corresponding frequency was 13.5 % (GRETTE 1948).

According to statistics of 1948, 1,672 fresh acquired syphilis cases were reported in Denmark, corresponding to the frequency

of 4.0/10,000; in the same period 34 new cases of congenital syphilis were reported.

Before 1910 Iceland was practically free from venereal diseases. In 1920 — 40 in this island of about 140,000 inhabitants an average of 25.5 new syphilis cases were reported each year. During World War II the number rapidly rose and the peak was attained in 1942, when 141 new syphilis cases were reported. Thereafter a decreasing tendency has been prevalent, and in 1947 — 48 the frequency of new reported cases was 0.6/1000 (Guðmunsson 1951).

Among the Eskimoes of Greenland syphilis is said to be almost unknown. When investigating a gonorrhea epidemic in Greenland Marcussen and Rendal (1949), however, found 5 syphilis cases in a village of 153 inhabitants.

In Cologne 18,436 persons were examined serologically in 1947 — 49. 5,878 of these had either fresh or latent syphilis (Büttgenbach 1951). According to some statistics, during World War II about 100 new syphilis cases were reported monthly in Berlin, after the war the number increased so that in 1946 about 15,000 new syphilis cases were reported in Berlin.

In 1942 Ten Berge arrived at the conclusion, on the basis of his calculations, that 2 % of the inhabitants of the Netherlands were affected by syphilis. During the war the number of syphilis cases in the Netherlands increased noticeably. Thus according to Groen (1948) in a province of the Netherlands two new syphilis cases were reported in 1938; in 1944 the corresponding number was 334, and ever since this number has remained high. In 1948 a total of 4,500 new syphilis cases came under treatment (Prakken 1949).

Milgrom (1948) from a material covering 113,845 blood samples estimated the syphilis frequency in Poland to be 0.67 %. The highest frequency — 3.86 % — was among the personnel of business enterprises and hotels. Lebioda (1948) examined serologically 17,347 of 20,007 Polish university students, the syphilis frequency being 3.5/1000, and he presumes that the frequency would have increased to 5 — 7/1000 if all the students had been investigated.

Ostertag (1950) mentions that in Bosnia and Herzegovina syphilis is endemic. The syphilis frequency is 10 — 15 % of the examined, in some villages it might even rise to 100 %. From

Hungary FEJÉR (1948) reports that in 1937 — 39 in St. Stephan Hospital only 3 children with acquired venereal disease were treated, in 1945 — 47 there were 65, of whom 35 had syphilis. The generalization of extragenital infection, is, in his opinion, due to poor hygienic circumstances. In Greece the frequency of reported syphilis cases was 6 — 8/1000 in recent years (DOUCAS 1950). According to the information of the WHO (1951) 9.6 % of the material covering 15,631 expectant mothers examined in Italy in 1950 had strong positive seroreactions for syphilis, and 2.2 % doubtful reactions.

SIMON (1948 *a*) holds the opinion that 20 % of French men have had syphilis infection. In LARIB's (1945) material of 1,769 stillbirths congenital syphilis was concerned in 653 cases. According to him the syphilis mortality for children of 0 — 1 year of age, was 3.8 % in towns and 2.7 % in the country.

In 1946 the frequency of venereal diseases cases in the English army, according to statistics, was 32.8/1000. In 1948 MACFARLANE analysed a serologic material comprising 40,000 pregnant women and 50,000 blood donors and came to the result that 1 % of the English suffer from syphilis.

In the United States of America VONDERLEHR and USILTON (1942) analysed a material of 1,895,778 samples serologically tested in 1940. These samples had been collected from men between the age of 21 and 35 years. On the basis of this material they found the syphilis frequency of negro men to be 252.3/1000 and that of white men 17.4/1000. The average age for syphilis infection is 22. ± 1.4 years for U.S. negro women and 25.1 ± 0.4 years for negro men, and for the white population the corresponding figures are 25.6 ± 1.6 years and 29.5 ± 0.6 years respectively (FRAZIER 1948). When 500,000 Georgians (U.S.A.) were examined serologically, positive results were obtained from 5,600 (BOWDOIN and BUCHANAN 1949). In the state of Alabama (U.S.A.) in 1943 all persons aged 14 — 50 years were examined serologically. There were 6.5 % positively reacting. For the white population this figure was 1.25 % and with negroes 17.14 % (SMITH and GILL 1950). BIBLER and GOLEY (1947) examined 42,103 soldiers in a demobilization center of the U.S. navy; 1.2 % had seropositive syphilis. ORR and RENTIERS (1951) report a serologic test in North Alberta (U.S.A.), in a very sparsely populated region of 1,300 Red

Indians of whom 145 had positive seroreactions. These 145 Red Indians were given a control examination in which 139 proved to remain positively reacting; from 8 of them a positive result was obtained with cerebrospinal fluid also. LAIN (1951) reports his examination of thousands of Indians in the years 1902 — 1915 when hardly any syphilis was found. He is of the opinion that negroes have spread syphilis infection among the Indians. In New York City 261/10,000 new syphilis cases were reported in 1949 (DROLET 1951). In the same year the congenital syphilis mortality in the whole of the U.S.A. was 9 per 1000 live births; in the same year 3.2 patients per 100,000 inhabitants were admitted to mental hospitals on account of syphilis (WRIGHT 1951). WILLCOX (1949) calculates that there are in the U.S.A. 3.2 million people suffering from syphilis.

As examples of the syphilis frequency in South America it may be noted that in 1946 1.8 ± 0.3 % of the expectant mothers in Cordoba had seropositive syphilis, in 1934 the figure had been 7.8 ± 0.9 % (CHATTAS 1950). From Uruguay GARZON (1950) reports that to-day 3 — 4 % of expectant mothers are syphilities, while the congenital syphilis frequency is 3.7 % of births.

In Hawaii 1.5/1000 new syphilis cases were reported in the year 1945 (ALLISON 1947).

Of the population of South Africa, depending on the district, 2.0 — 46 % suffer from syphilis (KARK 1949). For instance, in Uganda 25.7 % of the patients of a maternity ward were Kahn positive. However, only 1.5 % — 5 % of the children in Uganda were congenital syphilities (MUWAZI, TROWELL and DAVIES 1947). In Masai one in five inhabitants has syphilis (MCKAY 1950). Of 822 unselected Bantu tribesmen 17 % had positive WR, primarily either late syphilis or latent syphilis (O'MALLEY 1950). In Abyssinia 16.4 % of 462,608 patients seeking hospital treatment had syphilitic diseases. The Kahn positivity varies in the different provinces of Abyssinia from 42.9 to 95.5 % of the population (GUTHE 1949).

In Palestine 0.34/1000 new syphilis cases were reported in 1947; of the 8000 emigrants arriving in Palestine in 1946 — 47 120 had certain syphilis and 40 were syphilis suspects, the majority of these syphilities were from Roumania (FEILCHENFELDT 1949). In Afghanistan about one half of 5,000 patients examined had

syphilis (CUTLER 1950). In India there were in 1948 13 million people suffering from syphilis and gonorrhea, or 3.7 % of the whole population. There were 10 gonorrhea cases for every 17 syphilis cases (RAJAM 1948). In Ceylon, where there were 5.5 million inhabitants in 1938 2,527 patients were treated for syphilis during that year. In Burma, in 1939, 39,791 patients were hospitalized on account of syphilis. In China 6.4 % of hospital patients are in hospital for venereal disease treatment. In North China, of the material serologically investigated for blood donation 10 % proved to be seropositive. 40 % of the inhabitants of Korea are said to have syphilis. In Japan there are 10 times more venereal diseases than among the white population of the U.S.A. In Thailand 4,556 of the 13,042 blood samples examined in 1936 were Kahn positive. The yaws frequency in Thailand at the same date was 6.6 % of the investigated. Yaws, in fact, is very general in these East Asiatic territories; in the Dutch East Indies the yaws frequency is in some places said to amount to even 80 — 90 %. In New Caledonia 47 % of 892 investigated blood samples were WR-positive. In the New Hebrides syphilis is almost unknown among the natives, while the yaws frequency is 77 %. All the above information concerning East Asia and the archipelago is presented according to SIMMONS, WHAYNE, ANDERSON and HORACK (1944).

If we consider this scattered information on syphilis frequency in the different parts of the world, it might be partly agreed with ANDERSON *and* ARNSTEIN *(1948) that syphilis and civilization go hand in hand. The present material on the other hand seems to indicate that a high civilization level and developed social conditions are almost throughout connected with a low syphilis frequency.*

FEATURES OF THE STUDIES OF THE INCIDENCE OF SYPHILIS IN FINLAND

The information concerning the incidence of venereal diseases in Finland up to 1849 has been collected by RABBE (1849 — 50). The pre-1849 data in the following are based on RABBE's investigations. The first item of information about venereal infection in

Finland is available from the minutes of the diocesan council for 1641, 1661 and 1670, in which three sporadic cases of "French disease" — "Morbus Gallicus" or "Fransose" are mentioned. For 1729 36 cases are reported from Vaasa, and in 1755 a public institution for treatment — a "Curhus" was established at Hämeenlinna and Helsinki. In 1785 the venereal diseases are mentioned as general in the whole country as a consequence of the Pomeranian War. In 1756 the District Physician Institution was established, the duties of which included the prevention and control of the spreading of the venereal diseases. In 1783 the Collegium Medicum inquired the district physicians' opinion regarding venereal diseases. All of them held the opinion that venereal diseases were very general and would require special measures. The Collegium Medicum considered that war and foreign trade increased these diseases and suggested that the crews of all ships arriving in the contry should be examined, that soldiers should also be examined, and that all maidservants, vagabonds, etc. should always carry with them a health card. In spite of these measures the infection spread and in 1800 the first cases were reported from among the Lapps. In 1807 it was reported from the regions of Kuopio that venereal diseases appeared in almost every village. In 1800 — 1806 in the County Hospital of Turku a total of 996 patients were treated, of these about 36 % suffered from venereal diseases (Kalliomäki 1952). During the years 1816 — 1825 in the "Curhus" institutions mentioned above a total of 12,432 patients were treated. In 1831 — 1848 again the County Hospitals treated a total of 38,670 patients suffering from venereal diseases. The proportion of syphilis among the patients treated at that time can be seen from the fact that for example in 1848 in the County Hospital a total of 5,078 patients were treated, including 2,701 admitted to hospitals because of syphilis. According to Karvonen (1906) in 1846 — 1855 in the hospitals of Finland an annual average of 2,591 syphilis patients were treated, in 1856 — 1865 the corresponding figure was 3,704. Hjelt (1873) analysed in greater detail the syphilis material treated in all Finnish hospitals during the years 1859 — 1870. He obtained an incidence figure of 1.38/1000 for the whole country; in the countryside the figure was 1.11/1000 and in towns 4.14/1000. In the County of Turku the incidence was 1.57/1000 and in the town of Turku 4.46/1000. To take the year 1870 as an example: in this

year 8,629 Finnish people, or 0.5 % of the total population were in venereal disease hospitals for over one month, the number of other patients was 9,539 in the same year. This must be considered as an aftermath of the previous years of famine, when a large section of the population was compelled to wander from place to place in search of bread (KARVONEN 1906) LEONTJEFF (1900) made a careful study of the syphilis material treated in Finnish hospitals in 1877 — 1897. He assessed the syphilis incidence at this time at 1.26/1000. Most syphilis was found in the Counties of Mikkeli, Kuopio and Häme, the County of Turku and Pori held the 6th place with an incidence of 1.06/1000. Of the population centres the town of Mikkeli came first with an incidence of 6.74/1000. According to KARVONEN (1921) an average of 0.88/1000 syphilis patients were treated in the hospitals of Finland in 1890 — 1914. This figure is arrived at when the number of hospital patients is converted to an incidence figure for the whole country. OLIN (1939, 1941) examined Finnish syphilis statistics for 1916 — 1940. During this time two peaks appeared in the syphilis curve. The first peak succeeded World War I, and the second occurred around the year 1930, when 6.6/1000 new syphilis cases were reported. This second peak OLIN considers to be due to the economic depression prevailing at that time. When considering the venereal disease situation in Finland during and after World War II, PUTKONEN (1950) observed two syphilis waves proper during this time. The situation was worst in 1943 when 140.1/100,000 new syphilis cases were reported. This peak PUTKONEN attributes to the quiet period at the front, which allowed the men more leave. Another peak in the syphilis curve was reached in 1946 (124.1/100,000), since when the number of reported syphilis cases has continued to decrease throughout the country so that in 1950 Finland was on the whole on the same level with Sweden in this respect, according to PUTKONEN, the incidence figure then being 9/100,000. The proportion of congenital syphilis in Finnish official statistics was in 1934 — 48 an average of 69 children a year; 13 cases of syphilis other than congenital in children were reported during the same time (HÄRÖ 1950). In the years 1930 — 49 140 — 266 patients annually were taken in for the first time for treatment for insanity of syphilitic origin. In 1949 the figure was 174 (HÄRÖ 1951).

Several syphilis incidence investigations have been carried out by serodiagnostic means in Finland. TURUNEN (1935) analysed the serologic material of 5,092 mothers who had consulted the Maternity Welfare Center of the Women's Clinic of Helsinki, and found that 3.8 % had had syphilis in seropositive stage. In MAIJALA's (1939) material of factory workers who assessed their own condition as healthy, the WR was positive in 1.3 % and the Kahn in 2.1 %. HONKANEN (1937) investigated 611 pulmonary tuberculotics and detected seropositive syphilis in 5.2 %. In MAIJALA's (1942) dissertation material, comprising 10,954 patients of the Medical Clinic, seropositive syphilis was found in 3.28 %. KILPIÖ, PUTKONEN and PÄTIÄLÄ (1948) examined 10,445 university students, of whom 0.08 ± 0.1 % had seropositive syphilis of which they themselves were unaware. SETÄLÄ (1948) assessed the syphilis incidence for the inhabitants of the completely rural Kärkölä commune at 1.77 %. In LEINEBERG's (1949) material of 5,684 factory workers 1.09 % showed positive seroreactions for syphilis. In the Finnish blood donor material the incidence of seropositive syphilis was 1.2 % (PUTKONEN and PÄTIÄLÄ 1950). In 1944 — 1947 5.1 % of the personnel of the restaurants in Helsinki were suffering from syphilis in seropositive stage. (PÄTIÄLÄ and TALVITIE 1951, PÄTIÄLÄ 1951). Of the 74,655 pregnant women examined in 1949 0.97 % showed positive seroreactions for syphilis (PUTKONEN 1951 *a*).

To describe briefly the course of syphilis infection in Finland, it may be said that syphilis infection evidently came to Finland in the 17th century, in the 18th and 19th centuries syphilis was very widely spread in Finland and presented a considerable menace to the national health. In the 20th century World War I, the economic depression of the 1930's and World War II caused three serious peaks in the syphilis curve. Since World War II the situation, however, has been improving rapidly, so that in 1950 there were as few new syphilis cases reported as in Sweden.

PRESENT OBSERVATIONS ON SYPHILIS INCIDENCE

Subjects dealt with

The present study of syphilis incidence in the sphere of activity of the Department of Bacteriology and Serology of the University of Turku — i.e. in Turku and Pori County — is an attempt

— to ascertain, on the basis of the official data and the seropositive syphilis material of 1946 — 1950 of the Department of Bacteriology and Serology of the University of Turku the distribution of syphilis cases by territory, occupation and age class in Turku and Pori County.
— to ascertain, on the basis of the seropositive material, the syphilis frequency among expectant mothers, prisoners, alcoholics and Poor Law Institute dependents.

The Territorial Distribution of Syphilis Cases in the County of Turku and Pori

The County of Turku and Pori covers a territory of 22,003.12 sq. kilometres in the west-south-west corner of Finland. The territory is roughly rectangular in form, the south and west sides of the rectangle being bounded by the sea, the north and east sides by land. There were 611,316 inhabitants in this territory in 1948, of whom about 151,000 were living in towns, about 19,000 in market towns and about 441,000 in rural communes. There are 5 towns in the county, an equal number of market-towns and 108 rural communes. There is no information available, as yet, on the distribution of occupations among the population of this territory. By far the most important sources of livelihood in the rural communes, however, are agriculture and forest management in their different forms, only in a few rural communes on the coast is fishing more important than these. The sources of livelihood for the inhabitants of towns and market towns are of course chiefly industry, trade and communications, together with various kinds of intellectual and administrative work. In the country, factory and mining installations are found at Eura, Harjavalta, Hämeenkyrö, Kemiö, Kisko, Parainen, Ulvila, Vehmaa and Viljakkala.

Table 39 shows the territorial distribution in the County of Turku and Pori of the new syphilis cases reported in the years 1947 — 50 and of the seropositive syphilis cases of 1946 — 50 of the Department of Bacteriology and Serology of the University of Turku originating in the county. Owing to the fact that the regular submission of all samples from the county to the Department of Bacteriology and Serology of the University of Turku was not introduced until 1949, the seropositive syphilis cases derive to a large extent from the years 1949 — 50. Thus the syphilis cases of the City of Turku and its hospitals will constitute a great majority of the whole seropositive syphilis material for 1946 — 50

of the Department of Bacteriology and Serology of the University of Turku, because the samples before 1949 came primarily from Turku alone. From Laitila also samples were sent to our laboratory during the whole period 1946 — 50. The domiciles of the hospital patients included in the seropositive material were noted from the journals; others have been grouped according to the place of practice of the physician who has sent the sample. Thus certain potential errors are involved. Because of the obligation of secrecy imposed by the Lex veneris, however, the checking of the results presented insurmountable difficulties. However, these potential errors will hardly involve any essential changes in the territorial distribution. The officially reported new syphilis cases for 1947 — 50 have been totalled. This four-year period was chosen so as to correspond to the period of 1877 — 80 taken as the object of comparison. The frequency number in Table 39 illustrating the last mentioned period has been computed by LEONTJEFF (1900) and it has been reached on the basis of the material covering the patients then under treatment for syphilis in all hospitals in Finland. As the territorial correlations of gonorrhea and syphilis are of a certain interest, the numbers of the gonorrhea cases in 1947 — 50 for each locality in the County of Turku and Pori have been collected from official reports. Because of the present very "acute" character of gonorrhea, due to effective treatment, the numbers of the cases reported each year have been given separately also. The populations of the localities are based on the "Suomen Kunnalliskalenteri" for 1949. When computing the incidence the figures for populations have been rounded to the nearst thousand or five hundred.

Table 39.

Territorial Distribution of Reported and Seropositive Syphilis Cases in the County of Turku and Pori as Compared with the Syphilis Situation in the Years 1877 — 90 (LEONTJEFF 1900) and the Gonorrhea Situation in the Years 1947 — 50

Place	Number of Inhabitants in 1948—1949	Reported Syphilis Cases in 1947 — 50		Seropositive Syphilis Cases in 1949 — 50		Syphilis Frequency ‰/yr. in 1877—80	Reported Gonorrhea Cases in 1947 — 50				
		Absol. No.	Frequ. ‰	Absol. No.	Frequ. ‰		1947	1948	1949	1950	Total
Turku	93735	601	6.4	358 (1946 — 50) (2125	3.8 19.5)	3.05	1078	959	855	825	3717
Pori	39197	234	6.0	155	4.0	3.70	354	346	175	99	974
Rauma	12507	57	4.6	61	4.9	4.26	159	71	77	29	336
Uusikaupunki	3921	0	—	12	3.0	1.12	5	2	0	0	7
Naantali ...	1782	14	7.0	14	7.0	1.00	33	12	2	9	56
Ahlainen ...	3685	0	—	3	0.9	1.03	3	4	2	0	9
Alastaro ...	5624	5	0.9	18	3.4	4.30	2	2	5	5	14
Angelniemi .	1408	2	1.3	1	0.7	0.31	5	3	3	3	14
Askainen ...	1200	2	2.0	6	6.0	0.83	1	1	1	0	3
Aura	2772	0	—	2	0.7	1.00	0	0	0	2	2
Dragsfjärd .	4528	13	2.9	8	1.8	0.37	7	15	3	3	28
Eura	6556	9	1.4	19	2.9	0.54	26	6	3	7	42
Eurajoki ...	6445	8	1.2	6	0.9	0.71	18	12	6	1	37
Halikko	7151	10	1.4	11	1.6	0.24	12	11	9	2	34
Harjavalta .	5755	14	2.3	8	1.3	0.67	23	36	17	10	86
Hiittinen ...	1216	0	—	0	—	—	2	0	1	0	3
Hinnerjoki ..	1900	0	—	3	1.5	0.38	0	0	0	0	0
Honkajoki ..	3961	0	—	5	1.3	1.20	4	0	0	0	4
Honkilahti ..	1940	0	—	1	0.5	0.21	0	0	0	0	0
Houtskär ..	1418	0	—	1	0.7	—	0	0	0	0	0
Huittinen ..	10510	29	2.8	21	2.0	1.54	20	3	10	1	34
Hämeenkyrö .	10015	19	1.9	22	2.2	0.75	14	6	3	3	26
Ikaalinen ..	10934	13	1.2	5	0.4	0.69	33	21	9	7	70
Iniö	595	0	—	2	4.0	—	0	0	0	0	0
Jämijärvi ..	4011	5	1.3	5	1.3	1.31	4	4	1	3	12
Kaarina	4353	16	3.6	17	3.8	1.97	10	8	16	2	36
Kakskerta ..	758	0	—	1	1.0	1.78	2	3	2	0	7
Kalanti	3931	0	—	4	1.0	0.85	9	3	1	0	13
Kankaanpää .	11912	29	2.4	30	2.5	1.89	31	16	23	16	86
Karinainen .	2577	4	1.6	3	1.2	0.17	0	0	0	1	1

Place	Number of Inhabitants in 1948—1949	Reported Syphilis Cases in 1947 — 50		Seropositive Syphilis Cases in 1949 — 50		Syphilis Frequency ‰/yr. in 1877—80	Reported Gonorrhea Cases in 1947 — 50				
		Absol. No.	Frequ. ‰	Absol. No.	Frequ. ‰		1947	1948	1949	1950	Total
Karjala	1581	0	—	2	1.3	0.25	0	0	0	1	1
Karkku	3911	2	0.5	2	0.5	1.80	3	2	0	0	5
Karuna	1604	0	—	7	4.7	—	0	0	0	0	0
Karvia	5362	3	0.5	4	0.7	0.10	0	0	0	0	0
Kauvatsa ..	3238	5	1.7	2	0.7	0.83	4	3	2	0	9
Keikyä	2347	14	5.6	4	1.6	—	8	1	0	0	9
Kemiö	6480	11	1.7	24	3.7	0.32	7	8	7	4	26
Kihniö	4256	1	0.2	6	1.3	—	1	1	8	0	10
Kiikala	4071	2	0.5	3	0.8	—	6	9	1	0	16
Kiikka	4436	4	0.9	3	0.7	1.70	8	2	2	3	15
Kiikoinen ..	2870	4	1.3	3	1.0	2.65	1	6	8	0	15
Kisko	3996	4	1.0	9	2.3	1.30	4	0	1	6	11
Kiukainen ..	5706	0	—	6	1.1	0.47	11	3	8	4	26
Kodisjoki ..	736	0	—	0	—	—	0	0	0	0	0
Kokemäki ..	10552	15	1.4	19	1.8	2.54	20	25	5	11	61
Korppoo ...	2048	0	—	11	5.5	0.65	0	0	0	1	1
Koski	4207	4	1.0	11	2.8	1.20	4	0	2	0	6
Kullaa	2976	3	1.0	3	1.0	3.48	4	3	0	0	7
Kustavi	2088	2	1.0	5	2.5	—	1	4	2	2	9
Kuusjoki ...	2823	1	0.3	5	1.7	—	12	0	0	3	15
Köyliö	4443	1	0.2	2	0.4	0.65	1	6	0	0	7
Laitila	9523	24	2.5	26	2.8	1.37	9	10	7	3	29
				(1946 — 50)							
				62	6.5						
Lappi	3874	2	0.5	7	1.8	0.10	0	0	0	0	0
Lavia	5614	6	1.1	10	1.8	4.79	3	8	5	1	17
Lemu	952	1	1.0	0	—	—	1	0	1	0	2
Lieto	5236	1	0.2	17	3.4	3.53	0	3	11	0	14
Loimaa											
market town	3242	26	8.7				9	3	7	18	37
rural commune	9832	7	0.7	34	2.6	3.39	1	7	6	16	30
Lokalahti ..	1869	2	1.0	4	2.0	0.33	6	1	1	0	8
Luvia	3156	3	1.0	5	1.7	0.52	7	0	8	0	15
Maaria	1301	2	1.3	8	5.3	1.44	3	1	5	0	9
Marttila	3490	3	0.9	20	5.7	0.17	3	2	2	4	11
Masku	1915	0	—	6	3.0	1.73	0	0	2	0	2
Mellilä	2916	4	1.3	7	2.3	—	4	2	0	1	7
Merikarvia ..	7501	15	2.0	16	2.1	1.27	17	16	13	7	53

Place	Number of Inhabitants in 1948—1949	Reported Syphilis Cases in 1947 — 50		Seropositive Syphilis Cases in 1949 — 50		Syphilis Frequency ‰/yr. in 1877—80	Reported Gonorrhea Cases in 1947 — 50				
		Absol. No.	Frequ. ‰	Absol. No.	Frequ. ‰		1947	1948	1949	1950	Total
Merimasku ..	886	2	2.0	1	1.0	0.55	3	1	0	0	4
Metsämaa ..	2175	2	1.0	6	3.0	—	0	2	0	4	6
Mietoinen ..	3157	0	—	7	2.3	0.17	4	0	0	2	6
Mouhijärvi .	4665	2	0.4	4	0.9	2.03	1	0	0	0	1
Muurla	1839	4	2.0	0	—	0.68	9	3	0	0	12
Mynämäki ..	5125	2	0.2	17	3.4	0.07	7	2	3	3	15
Naantali rural commune	1533	1	0.7	0	—	1.87	2	2	0	1	5
Nakkila	6648	14	2.2	16	2.5	0.66	0	16	15	5	36
Nauvo	2500	4	1.6	11	4.4	0.25	3	7	2	0	12
Noormarkku .	4555	7	1.6	14	3.1	0.40	2	2	2	0	6
Nousiainen .	3371	1	0.3	4	1.1	0.09	8	4	3	4	19
Oripää	2364	0	—	8	3.2	0.42	0	0	0	0	0
Paattinen ..	1299	3	2.0	2	1.3	—	2	3	3	2	10
Paimio	7037	6	0.9	29	4.1	0.74	1	3	9	3	16
Parainen market town	5478	33	6.0	61	5.8	0.71	53	52	35	41	181
rural commune	4928	5	1.0				?	16	18	5	39
Parkano ...	8818	10	1.1	12	1.3	0.94	15	14	8	2	39
Perniö	9935	15	1.5	18	1.8	1.15	24	11	8	3	46
Pertteli	3766	4	1.0	5	1.3	0.43	17	15	1	2	35
Piikkiö	3620	9	2.6	12	3.4	0.21	4	10	4	6	24
Pomarkku ..	4825	2	0.4	8	1.6	0.83	5	2	6	1	14
Pori rural comm.	6608	23	3.5	16	2.5	3.69	9	7	11	7	34
Punkalaidun	8696	7	0.8	24	2.8	2.17	10	12	3	2	27
Pyhämaa ...	1297	0	—	1	0.7	—	0	0	0	0	0
Pyhäranta ..	2756	0	—	4	1.3	—	0	2	1	0	3
Pöytyä	5432	0	—	7	1.3	1.28	0	8	1	6	15
Raisio	4086	13	3.3	12	3.0	0.47	6	10	8	1	25
Rauma rural commune	7476	9	1.2	?	?	0.10	0	12	11	10	33
Rusko	1050	0	—	2	2.0	—	0	1	0	3	4
Rymättylä ..	2901	5	1.7	10	3.3	0.57	4	11	0	2	17
Salo market town	8780	14	1.6	68	6.2	—	51	35	31	9	126

Place	Number of Inhabitants in 1948—1949	Reported Syphilis Cases in 1947 — 50		Seropositive Syphilis Cases in 1949 — 50		Syphilis Frequency ‰/yr. in 1877—80	Reported Gonorrhea Cases in 1947 — 50				
		Absol. No.	Frequ. ‰	Absol. No.	Frequ. ‰		1947	1948	1949	1950	Total
Sauvo	3349	0	—	24	6.9	0.09	0	1	4	2	7
Siikainen ..	5171	8	1.6	14	2.8	4.63	4	2	0	0	6
Suodenniemi .	3064	4	1.3	1	0.3	—	2	4	0	0	6
Suomusjärvi .	2142	1	0.5	2	1.0	2.50	0	0	0	0	0
Suoniemi ...	2491	1	0.4	0	—	0.95	0	1	2	0	3
Säkylä	3328	1	0.3	6	1.7	0.42	3	1	1	1	6
Särkisalo ...	2363	2	0.0	0	—	0.42	4	4	0	0	8
Taivassalo ..	3109	3	1.0	7	2.3	0.60	1	3	0	0	4
Tarvasjoki .	2500	2	0.8	3	1.2	—	1	1	1	0	3
Tyrvää (+ Vammala) .	10238	5	0.4	11	1.0	1.57	32	13	11	8	64
Ulvila	6129	12	2.0	15	2.5	2.44	8	13	9	3	33
Uskela (see Salo) .	2525					0.52					
Uusikaupunki rural commune	1329	1	0.7	0	—	1.25	0	0	0	0	0
Vahto	1208	5	5.0	5	5.0	0.36	0	0	0	0	0
Vammala market town (see Tyrvää)	1100										
Vampula ...	3845	2	0.5	4	1.0	1.00	5	4	1	2	12
Vehmaa	4659	14	3.1	19	4.2	0.16	7	5	2	2	16
Velkua	363	1	2.0	1	2.0	—	1	0	1	0	2
Västanfjärd .	1645	8	5.3	1	0.7	—	1	2	0	0	3
Viljakkala ..	2815	6	2.0	12	4.0	0.50	3	1	1	2	7
Yläne	3828	0	—	10	2.5	0.31	0	0	0	3	3
Total	611316	1548	2.5	1607	2.6	1.31	2325	1988	1554	1260	7127

On the basis of the information given in Table 39 only a few general conclusions can be drawn. It is found that the numbers of new reported syphilis cases and seropositive syphilis cases show the same trend, however, so that it seems that there are generally more seropositive cases in most places. This naturally is partly due to the fact that old, already reported cases move

from one place to another. This is partly supported by the fact that in the incidence figures for the whole county there is no great difference between the new reported cases and the seropositive cases. If we compare the present syphilis incidence figures with the figures for years 1877 — 80 we can observe that they generally run parallelly, of course with numerous exceptions. It would appear that this is due, in the first place, to the fact that the economic geography of the county has not undergone any major changes during the 70 years under examination: the most important thoroughfares and economic centers have remained in the same places, on the whole. If we wish to compare the incidence figures for the years 1877 — 80 and 1947 — 50 quantitatively, the figures for the years 1877 — 80 must first be multiplied by 4. The best general picture of these quantitative proportions will then be obtained by comparing the incidence figures for the whole province. The "converted" incidence figure for the years 1877 — 80 will be more than double the incidence figure for the years 1947 — 50.

A comparison of the territorial distribution of the syphilis and gonorrhea cases on the basis of Table 39 gives the general impression that where syphilis infection is general there are also more gonorrhea cases. This is no rule, however, and especially in the country disproportion is found in many places between the number of syphilis cases and that of gonorrhea cases. From Table 39 one may draw the conclusion that gonorrhea is a disease which is still more connected with town-dwelling than syphilis. Table 40 is an attempt at a more detailed illustration of this point. It gives the distribution of the syphilis and gonorrhea cases reported in 1947 — 50 for the towns and market towns and for the rural communes.

According to Table 40 64.4 % of the new reported syphilis cases were from towns and market towns, while the corresponding percentage for gonorrhea cases was 78.1. The difference between these two percentages is thus 13.7 ± 1.3, which can be regarded as statistically definite. This difference may partly be due to the fact that all the towns in this county are seaports, and gonorrhea especially is to a great extent "import goods" as it was possible to show with regard to Kotka, perhaps the most typical port in Finland, when the arrivals of ships there and the incidence of

Table 40.

Distribution of the Syphilis and Gonorrhea Cases Reported in the Years 1947 — 50 for Towns and Market Towns and for Rural Communes

Type of population center	Reported new Syphilis Cases		Reported new Gonorrhea cases	
	Absolute number	Percentage	Absolute number	Percentage
Towns and Market Towns (ca. 170,000 inhabitants)	997	64.4	5,568	78.1
Rural Communes (ca. 441,000 inhabitants)	551	35.6	1,559	21.9
Total (ca. 611,000 inhabitants)	1,548	100.0	7,127	100.0

new cases of venereal diseases were compared (PÄTIÄLÄ and TALVITIE 1951, PÄTIÄLÄ 1951). The fact that between 1946 — 49 10.5 % of the male gonorrhea patients in Finland were seamen also supports this view. In these years gonorrhea was still on the increase among seamen, although the curve illustrating the incidence of gonorrhea in the whole of Finland was decreasing (PUTKONEN 1951 *b*).

Classification of Seropositive Syphilis Cases According to Different Stages of Syphilis

A material covering 818 seropositive syphilis cases for the years 1946 — 50 and originating in the countryside of the County of Turku and Pori has been available for this study. These cases represent a total of 65 localities. Table 41 gives the classification of this material into different syphilis stages.

It is presumable that in the group of 205 cases in which the stage of the disease had not been reported, the majority of the cases are likely to be distributed between the secondary and latent syphilis. The majority of the official physicians from whom the information has been received classify the primary, tertiary and congenital syphilis cases, but for lack of objective criteria, often leave unsolved whether the seropositive syphilis is to be included

Table 41.

Classification of a Material Covering 818 Seropositive Syphilis Cases according to Different Stages of Syphilis

Stage of Syphilis	Number of cases	Percentage
Primary Syphilis	20	2.4
Secondary Syphilis	219	26.8
Latent Syphilis	256	31.3
Tertiary Syphilis and Neurosyphilis	71	8.7
Congenital Syphilis	47	5.7
The stage of the disease not reported	205	25.1
Total	818	100.0

in the secondary or latent syphilis group. In Finland in the years 1877 — 80 there were five early syphilis cases (primary syphilis and secondary syphilis) for every tertiary syphilis case (LEONTJEFF 1900). The number of tertiary syphilis cases, therefore, has decreased considerably in proportion. The difference would evidently be still greater had the diagnostic potentialities in 1877 — 80 been equal to those of the present day.

Classification of New Syphilis Cases Reported in the Years 1947 — 50 in the County of Turku and Pori by Occupation

In Table 42 the author has collected information about the distribution of the new syphilis cases reported in the years 1947 — 50 in the County of Turku and Pori according to occupation in accordance with the formula presented in the official blank forms of the Medical Board for reporting new cases of venereal diseases. The table also gives the changes in the occupational structure of the reported syphilis material during the period of the time under examination.

Figure 3 presents in graph form the yearly changes in the occupational structure of the syphilis material.

It is not possible to draw many conclusions on the basis of Table 42 about the syphilis frequency in different occupational groups in the County of Turku and Pori, because there is no

Table 42.

Classification by Occupation of the New Syphilis Cases Reported in the County of Turku and Pori and the Changes in this Occupational Structure during the Years 1947 — 50

Occupation or Trade	1947		1948		1949		1950		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Agriculture	83	12.8	55	11.6	55	20.1	22	14.3	215	13.9
Industry and Crafts	152	23.5	110	23.2	65	23.8	31	20.1	358	23.1
Traffic and Commerce	140	21.6	91	19.2	46	16.9	31	20.1	308	19.9
Public work and Professions	36	5.6	38	8.0	18	6.6	11	7.1	103	6.7
Other Trades ...	57	8.8	35	7.4	15	5.5	9	5.8	116	7.5
Unskilled workers and day-labourers	130	20.1	114	24.1	55	20.1	34	22.1	333	21.5
No Occupation ..	49	7.6	31	6.5	19	7.0	16	10.4	115	7.4
Total	647	100.0	474	100.0	273	100.0	154	100.0	1548	100.0

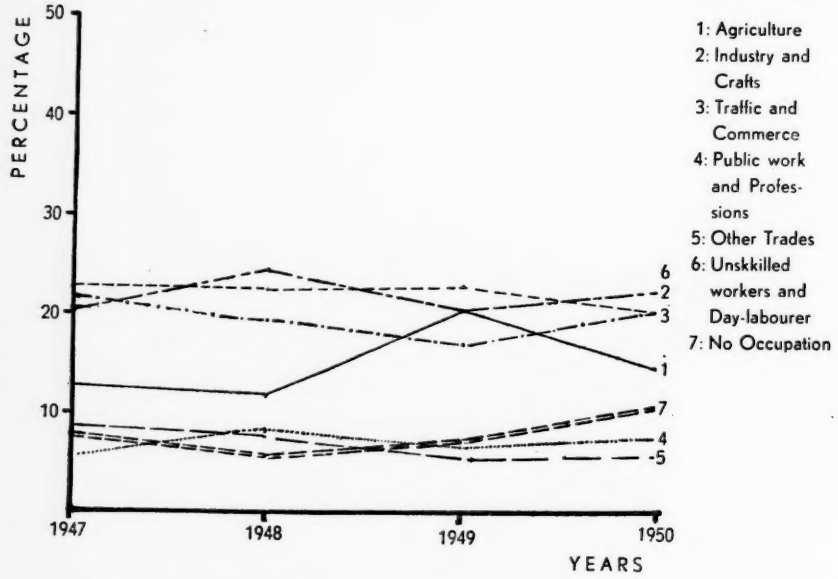


Figure 3. Percentual Classification by Occupation or Trade of the New Syphilis Cases Reported in the County of Turku and Pori in 1947 — 50

information available regarding the percentage of these trade groups of the population of the territory concerned. However, it can be considered fairly certain that the percentage of the agriculture-group of the total reported syphilis cases is smaller than its percentage of the total population of the county. Similarly it can be said that those having no occupation or trade, in the first place vagrants of different types, provide a larger proportion of the syphilis material than their proportion of the population of the province of the county.

From Table 42 and Fig. 3 it can be seen that the occupational grouping of the reported syphilis cases has remained largely unaltered during the years 1947 — 50. All variations in the percentage figures fall within the limits of potential statistical errors.

Table 42 shows, however, from the total results for each year the development of the syphilis situation in the County of Turku and Pori during the years 1947 — 50 within the frame of reported new syphilis cases. In the course of these 4 years the number of reported new syphilis cases has decreased to a quarter of the 1947 figure.

Classification by Age Groups of New Syphilis Cases Reported in the Years 1947 — 50 in the County of Turku and Pori

Information regarding the distribution of the new syphilis cases reported in the County of Turku and Pori in the years 1947 — 50 by age categories is presented in Table 43 which has been made to correspond with Table 42.

Figure 4 presents in graph form the findings shown in Table 43.

In following, from Table 43 and Fig. 4, the percentage grouping of the new syphilis cases during the years 1947 — 50, certain changes can be observed. The percentage of older age groups is on the increase in the material under examination, while the percentage of the younger age groups is decreasing. If the situations in 1947 and 1950 are compared, however, a statistically significant change has taken place only concerning the age groups 15 — 19 years and $\geqq$ 50 years. Other changes fall within the limits of potential statistical error. As a marked correlation is found also in the other percentual changes, primarily in the age categories of acquired

Table 43.

Classification by Age Groups of the New Syphilis Cases Reported in the County of Turku and Pori in 1947 — 1950

Age Category	1947		1948		1949		1950		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
0 — 4 years ...	5	0.8	12	2.5	5	1.8	5	3.2	27	1.7
5 — 9 years ...	6	0.9	1	0.2	2	0.7	1	0.7	10	0.6
10 — 14 years ...	5	0.8	2	0.4	0	—	1	0.7	8	0.5
15 — 19 years ...	77	11.9	25	5.3	11	4.0	7	4.5	120	7.8
20 — 24 years ...	133	20.6	125	26.4	46	16.9	20	13.0	324	20.9
25 — 29 years ...	157	24.3	115	24.3	55	20.2	24	15.6	352	22.7
30 — 49 years ...	221	34.2	161	34.0	110	40.3	67	43.5	559	36.1
≧ 50 years ...	43	6.6	33	7.0	44	16.1	29	18.8	149	9.6
Total	647	100.0	474	100.0	273	100.0	154	100.0	1548	100.0

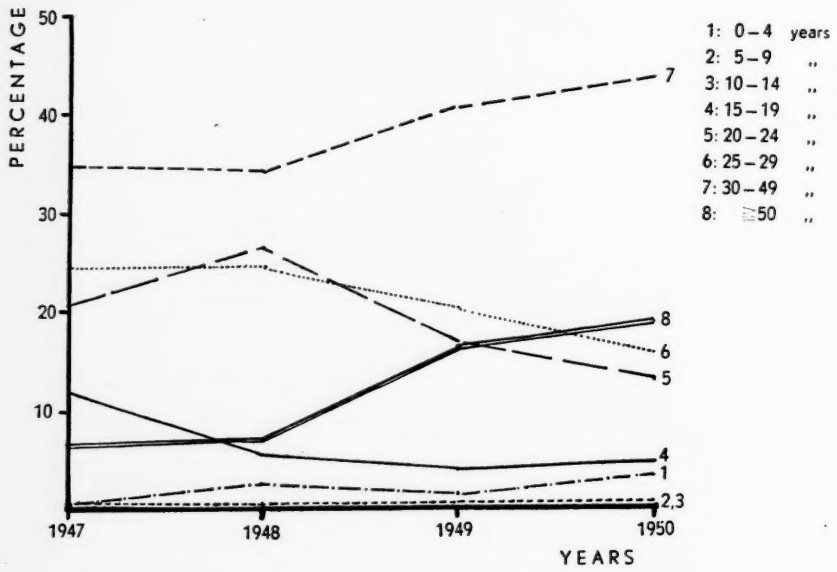


Figure 4. Distribution in Percentages of the Syphilis Cases Reported in the County of Turku and Pori in 1947 — 50 by Age Groups presented in Graph Form

syphilis, in so far as the proportion of the older age groups increases in relation to the younger age groups, the author would consider these changes also indicative of the true tendency, although the definite changes are to be found only in the youngest and oldest age categories. This result is correlated with the results earlier reached by the author when examining the age structure of the out-patient clinic syphilis material of the Dermatological Clinic of the University of Turku. In that material the average age of the syphilis patients rose by 10 years during the years 1946 — 50 (KALLIOMÄKI 1952). This increase of the percentage of the older age groups may be considered as a result of the decrease of syphilis, the "new" syphilis cases that are detected begin to be more and more often old latent cases. It is true that the age structure of the whole nation is changing toward the same direction as in this syphilis material, but this alone can hardly explain the change in the syphilis material in the course of such a short time.

The Frequency of Seropositive Syphilis among Pregnant Women in the County of Turku and Pori

The present observations concerning the incidence of seropositive syphilis in pregnant women are based on the material comprising 26,432 sera of pregnant women investigated in the Department of Bacteriology and Serology of the University of Turku during the period January 1, 1950 — October 11, 1951 partly reported in the serologic part of this study. The material was examined by the Kahn reaction, each suspicious Kahn result was checked and of the suspicious sera the WR and chol. WR also made. Positive results were obtained in a total of 421 sera, these samples derived from 258 mothers. For 142 mothers information about the specificity of the result was received. Of these 142 mothers 96 (67.6 %) were syphilitics according to the author's evaluation, and they had also been considered as such clinically, the remaining 46 mothers for whom details were available proved to be false positive. As according to the criteria presented in the serologic section, on the basis of the serologic status obtained by the reaction triangle WR, chol. WR, Kahn the activity of the disease can also be evaluated to a considerable extent. Table 44 gives results obtained with the

WR and chol. WR of the 183 Kahn-positive specimens collected from 96 syphilitic mothers.

Table 44.

The Number of Positive Results Yielded by the WR and chol. WR in the Material Comprising Explained Kahn-Positive Sera of Syphilitic Mothers

	WR	Chol. WR	Kahn
Positive	53	89	183
Negative	130	94	—
Total of specimens	183	183	183

The behaviour of the WR and chol. WR in the material under examination covering 171 Kahn-positive sera remained unexplained, has been shown in Table 29. Presuming that the proportion of syphilitic mothers of the unexplained part, (116 pregnant women) is equal (67.6 %) to their percentage of the explained part, the probable total of syphilitic mothers in the investigated materials is 174. It is the practice in the maternity advisory centers in Finland to take a single blood sample for serologic syphilis investigation. Such being the case, we can say that the 26,432 samples derive from about 26,000 pregnant women. The incidence of seropositive syphilis in the material is illustrated by the following table:

Serologically examined	26,000 pregnant women
No. of seropositive syphilities included among these	174
Incidence of seropositive syphilis in the investigated material of pregnant women	0.66 ± 0.05 %

The investigated material originates in the territory of the whole county of Turku and Pori and covers all classes of society in the proportion in which they appear in the population of this territory. Of the 96 expectant mothers, who according to the information received were established to be syphilitic, 89 had been given antisyphilitic treatment; about the possible treatment of 7 mothers no information was available. A stillbirth terminated pregnancy in 3 of these 96 mothers (in one of these cases a premature ablation of the placenta was involved).

Information was available on the postnatal blood test of the children of 25 syphilitic mothers delivered during the period of

the time under examination. Of these 8 had positive seroreactions. As the later results of these reactions are unknown, it is impossible to say whether a positive result caused by the mother's reagins, or a symptom of congenital syphilis was involved. The former possibility may be more probable with regard to the majority as the WR is reported to be positive in 2 children only. Even this examination may already indicate the great importance of prenatal serologic control and specific treatment, when necessary, in the fight against congenital syphilis. Thus ENKVIST (1949) is of the opinion that with adequate treatment up to 80 % of congenital syphilis can be prevented in Finland.

The number of earlier abortions of 72 syphilitic mothers and of 37 mothers from whose sera false positive Kahn-results were obtained is known. The results are to be seen from Table 45.

Table 45.

The Incidence of Earlier Abortions of Syphilitic Mothers and Mothers from Whose Sera False Positive Kahn-Results Were Obtained

	Syphilitic Mothers	False Positively Reacting Mothers
Number of Cases	72	37
Total of Abortions	22	2
Abortion Frequency per 100 mothers	31 ± 5.5	5 ± 3.5
Difference	26 ± 6.5	

With the help of the incidence figure presented in Table 45 it can be computed that probably 174 syphilitic pregnant women in the material under examination had 54 earlier abortions, while the number of abortions of an equal number of non-syphilitic women was 9. In the light of these figures the proportion of syphilis as the cause of the decrease of the birth rate can be estimated to some extent.

The explained material available of syphilitic mothers (96 mothers) consisted — as mentioned earlier — practically exclusively of syphilitic patients to whom antisyphilitic treatment had been given either during or before the pregnancy.

In order to be able to show whether the mother's treated syphilis affects the birth-weight of the child, Table 46 has been compiled

from the material. The birth-weight of the children of 90 syphilitic mothers is known. As control material the birth-weight of the children of the mothers whose sera yielded false positive Kahn-results (the mean value calculations are based on 40 cases) and the average birth-weights calculated by RITALA (1931) from a material covering children of I and II parae from Turku have been used. In the present material, the sex and the consecutive number of the child have not been taken into consideration in calculating the averages.

Table 46.

Comparison of the Average Birth-Weight of the Children of Treated Syphilitic Mothers and Healthy Mothers

	Group to which the Pregnant Women Belong			
	Syphilities	False Positive Kahn-results Obtained	Women from Turku	
			I-parae	II-parae
Average birth-weight of children in grams	3460 ± 55.0	3500 ± 100.0	3420 ± 25.0	3561 ± 28.9

From Table 46 it would appear that there is no significant difference in the birth-weight of the children of treated syphilitic mothers and those of healthy mothers.

To find out whether there is any difference in the distribution in sex of the children of mothers suffering from seropositive syphilis and the children of healthy mothers Table 47 has been compiled on the basis of the material. All infants born living or dead in Finland in the year 1949 have been taken as control material together with the children of the mothers whose sera yielded false positive Kahn-reactions during the pregnancy.

The material covering children of syphilitic mothers is unfortunately too small to justify definite conclusions. Among the percentages presented in Table 47 statistically definite differences exist only in the groups of infants born in Finland dead or living, in other words, the average proportion of males is higher among stillbirths than live births.

Table 47.

Distribution according to Sex of Children of Mothers Suffering from Seropositive Syphilis Compared with the Children of Mothers Whose Sera Yielded False Positive Kahn Results during Pregnancy and the Children Born Living or Dead in Finland in the Year 1949

	Children of Syphilitic Mothers	Children of Mothers Whose Sera Yielded False Positive Kahn-results during Pregnancy	All Children Born Living in Finland in 1949	All Children Born Dead in Finland in 1949
Total of Children ..	89	34	103,515	1,821
Male	52	17	53,276	1,025
Male in %	59 ± 5	50 ± 9	51.47 ± 0.16	56.3 ± 1.2

It is a generally known fact that an important cause of the death of the child in the uterus is maternal syphilis. Such being the case, it does not seem impossible that the percentages illustrating the proportion of male children obtained for the group of syphilitic mothers shows the true trend, in other words, the number of boys among the children of syphilitic mothers exceeds the average. The material under examination, however, does not give any conclusive reply to this question.

When analyzing his material comprising syphilitic mothers, PENTTINEN (1946) observed that 24.2 ± 2.5 % of them were unmarried, in the control material the corresponding percentage was 11 — 14 %. In the age grouping of the syphilitic mothers and the control material no differences were observed. 43.4 ± 4.3 % of the syphilitic mothers had something indicative of syphilis in the medical history of earlier pregnancies. In PENTTINEN's material there were no significant differences in the nephrogestosis frequency between the syphilitic mothers and the control material.

The Incidence of Kahn Positive Results in Prisoner Material

The author's observations on prisoners are based on the material investigated in the Department of Bacteriology and Serology of the University of Turku during the period January 1, 1950 — August 17, 1951 comprising 4.950 samples collected from prisoners. The

samples were derived from the following prisons: County Prison of Turku, Central Prison of Turku, Reserve Prisons of Huittinen, Karvia and Köyliö, Prison Colony of Leistilä and Prisoner Insane Asylum of Turku. A blood specimen is automatically taken from each prisoner on his arrival in the prison. In the laboratory the samples are first tested with the Kahn reaction. Where it yields a doubtful or positive result, the sample is retested on the following day with the WR, chol. WR and Kahn reactions, and this second test decides the result, and is the final classification. The following table shows the incidence of checked Kahn-positive cases in the prisoner material. The total of the examined prisoners was made a round figure of 4000 in order that any possible re-examinations of any one prisoner might be excluded with certainty. The potential error of the rounding falls on the decimal part of the percentage figure obtained.

Serologically investigated prisoners	ca 4000
Checked Kahn-positive cases among these	235
Percentage of Kahn-positive prisoners	5.9 ± 0.4

There are no facilities for re-checking the specificity of the checked Kahn-positive cases. Assuming that in the prisoner material the Kahn reaction yields as many false positive cases as in the present material covering pregnant women — 32 % of the seropositive cases — the frequency of seropositive syphilis in the prisoner material would be, thus corrected, 4.0 ± 0.3 %. If the percentage obtained is compared e.g. with the frequency number illustrating the seropositive syphilis of pregnant women — 0.66 ± 0.05 — reached on the basis of the present material, the difference must be considered as marked.

Table 48 gives the number of positive results yielded by the WR and chol. WR in the Kahn-positive prisoner material. On the basis of this table it can thus evaluated to some extent, whether the syphilitic prisoner material is composed chiefly of treated or untreated cases.

Table 48 shows that the WR and chol. WR positive cases are comparatively few in number, and thus the majority of the cases are evidently treated syphilis patients. This in fact is understandable as the prisoners are under severe serologic control and do not deject any opportunity of treatment given to them.

Table 48.

Number of Positive Results Yielded by the WR and chol. WR in the Kahn-Positive Prisoner Material

	WR	Chol. WR	Kahn
Positive	28	66	235
Negative	207	169	—
Total	235	235	235

Table 49 shows the distribution of the Kahn-positive prisoner material by the number of their terms of imprisonment. The relevant data were obtained for 206 prisoners.

Table 49.

Distribution of the Kahn-positive Material according to the Number of Times the Prisoner Has Been in Prison

Number of times in Prison	Number of Cases	Number of times in Prison	Number of Cases	Number of times in Prison	Number of Cases
I	42	VIII	8	XV	1
II	58	IX	6	Recidivist no exact information	6
III	13	X	3		
IV	12	XI	3		
V	16	XII	4		
VI	18	XIII	0	Not known	29
VII	16	XIV	0		

From Table 49 it may be seen that in the Kahn-positive material of 206 studied cases 42 were first offenders. The prisoners for the whole of Finland have been taken as a control. It is to be regretted that the most recent information available in this respect derives from the year 1947. All those sentenced to imprisonment or hard labour in 1947 have been included and divided into first offenders and recidivists. The comparison between this prisoner material for the whole of Finland and the present Kahn-positive prisoner material with reference to the proportion of first offenders in the whole material can be seen from Table 50.

Table 50.

Comparison of the Proportion of First Offenders in the Kahn-positive Prisoner

	Kahn-positive prisoner material	Prisoners in the whole of Finland for 1947
Total of prisoners	206	14,448
First offenders	42	5,833
„ „ in %	20.4 ± 2.8	40.4 ± 0.4
Difference between percentages	20.0 ± 2.8	

The difference of the percentages according to Table 50 is so clear that it is not possible to explain it from the fact that the materials are of different times and that one material covers the prisons in the County of Turku and Pori while the other covers all the prisons in Finland. In the quality of the offenders of different counties the differences can hardly be very great.

As to the type of the offences certain conclusions can be drawn regarding the character of the criminal. Table 51 shows how many prisoners in the Kahn-positive material revealed several types of crimes in their criminal registers. This information was obtained for 226 prisoners. Each type of offence has been included only once for each prisoner; on the other hand all the different types of crimes have been taken into consideration from the criminal register of each prisoner. The principal grouping of the crimes follows the grouping in the official criminal statistics of Finland.

The most recent information about the grouping of the total Finnish prisoner material by the type of offence was for the year 1949. Table 52 is a comparison between the breakdown into the main categories of different crimes of the Kahn-positive prisoner material and those sentenced to imprisonment or hard labour in Finland in 1949.

Table 52 shows that in the criminal register of the Kahn-positive prisoners there appeared fewer crimes against State or Society than in the prisoner material covering the whole country; regarding crimes against the Alcoholic Beverages Act the situation was entirely reversed. Crimes against individuals were equally numerous in both materials, the difference regarding the crimes against property is not statistically significant. Crimes not included in the

Table 51.

Incidence of Different Types of Offence in the Kahn-positive Prisoner Material

Type of Offence	Number of Prisoners in whose criminal register each crime appeared
A. Crimes against State or Society:	
Forgery	9
Violence against public officials	5
Sexual Offences	2
Total	16
B. Crimes against the Individual:	
Assault and Battery, Corporal Injuries and Negligent Manslaughter	15
Homicide and Assault leading to Death	2
Murder	1
Rape	1
Other Crimes of this Group	18
Total	37
C. Crimes against Property:	
Larceny, Petty Larceny Breaking and Entering	136
Fraud	18
Embezzlement	13
Receiving of Stolen Goods	10
Robbery	6
Total	183
Crimes against the Military Criminal Law	10
Crimes against the Alcoholic Beverages Act	64
Crimes against Rationing	11
Other Crimes	2
Total of Various Crimes in the Criminal Register of 226 Prisoners	323

Table 52.

Comparison between the Distribution of the Kahn-positive Prisoner Material and of Those Sentenced to Imprisonment or Hard Labour in Finland in 1949

Main Category of Crime	Kahn-positive Prisoners		Total Prisoner Material in Finland in 1949		Difference between Percentages
	Number	%	Number	%	
Crimes against State or Society	16	5.0 ± 1.2	1163	12.3 ± 0.3	— 7.3 ± 1.2
Crimes against the Individual	37	11.5 ± 1.8	938	10.0 ± 0.3	+ 1.5 ± 1.8
Crimes against Property	183	56.7 ± 2.8	4566	48.5 ± 0.5	+ 8.2 ± 2.8
Crimes against the Alcoholic Beverages Act	64	19.8 ± 2.2	512	5.4 ± 0.2	+ 14.4 ± 2.2
Other Crimes .	23	7.1 ± 1.4	2241	23.7 ± 0.4	— 16.6 ± 1.5
Total	323	100.0	9420	100.0	

above mentioned groups of crimes appeared in a considerably larger extent in the material representing the whole of Finland. This group of other crimes includes the crimes committed against the Military Criminal Law, crimes against rationing, offences not specified in the statistics as well as the cases in which the Ministry of Justice has declared the conditional liberty of an offender to be forfeited.

Thus, of the Kahn-positive prisoner material 80 % were recidivists and ca. 30 % of the Kahn-positive material studied had been in prison 4 or more times. In order to get a tentative picture of the nature of such a prisoner material from the medical and sociological points of view, it is perhaps permissible to compare the present material with the prisoner material of dangerous recidivists, which Ahto (1951) in Finland investigated and analysed from the psychopathological and sociological point of view. The material comprised 216 criminals. 8.8 % of the fathers of these criminals and 15.9 % of their mothers were mentally defective or insane, 54.4 % of their fathers and 42.8 % of their mothers were psychopaths. 48.6 % of the fathers were also alcohol addicts, 22.6 % were

vagrants and 29.7 % ex-convicts. For the mothers the corresponding percentage figures were 2.2, 12.7 and 14 respectively. Of the investigated criminals themselves 16.2 % were illegitimate children and 83.3 % had had miserable surroundings as children. 86.1 % of these investigated criminals had never had permanent work. 10.6 % of them were medically classified as alcoholics, sociologically this percentage rose to 64.3. The character traits of those investigated are summarized by AHTO as follows: "The characteristic features revealed were, in the order of prevalence, the following: restlessness, egocentricity, lack of willpower, instability, rashness, uncommunicativeness and irritability, emotional coldness and shallowness and self-emphasis."

In the literature few studies on syphilis incidence in prisoners are found, while studies on vagrants of different kind and the incidence of syphilis among them are more often published. MILGROM's (1948) Polish serologic mass examination material also included 1,336 prisoners, the seropositive syphilis frequency among them was 1.20 % and the percentage giving the syphilis incidence of the whole material 0.67 %. In French Guiana 22 % of 9,164 prisoners had positive seroreactions for syphilis. In addition it should be mentioned that among the prisoners there neurosyphilis was ten times more general than among free people (FLOCH and DELAJUDIE 1946). In LAGASSAGNE's (1948) material comprising 739 arrested persons 6.1 % had seropositive syphilis. DAHLBERG (1949) in his studies came to the result that those sentenced for drunkenness had a higher venereal disease frequency than the normal material.

KIRCHESCH (1950) investigated ca. 6000 woman and girl vagrants. ⅓ of them had some kind of venereal disease. In JOKIVARTIO's (1946) material of 100 woman vagrants 73 had syphilis. PÄTIÄLÄ and MÄKIKYLÄ (1948) analysed the vagrant material of Helsinki for the years 1937 — 47. Of male vagrants 17.7 % had suffered from some kind of venereal disease before being declared vagrants, for women the corresponding figure was 56.9 %. In each year covered by this study an average of 27.8 % of the vagrants suffered from venereal diseases. A good idea of the dangerousness of woman vagrants as spreaders of venereal diseases is given by a case published by PÄTIÄLÄ (1941) in which, during the war 27 soldiers caught syphilis from such a woman in two days. The occurrence

could have had more serious consequences, for in all 100 — 150 men had been in sexual intercourse with the same spreader of infection in the course of a few days.

The Incidence of Seropositive Syphilis in Alcoholics

The author's observations on alcoholics are based on the material investigated during the period January 1, 1950 — August 16, 1951 in the Department of Bacteriology and Serology of the University of Turku, covering 295 alcoholics. 251 of these were from Perniö Home for Alcoholics and 44 from Rymättylä Home for Alcoholics. The samples were investigated by the same system as has been reported in connection with the pregnant women and prisoners. By means of inquiries false positive results have been eliminated from the material. The following Table shows the incidence of seropositive syphilis in the investigated alcoholic material.

Serologically investigated alcoholics	295
Seropositive syphilis found among these	10
Percentage of seropositive syphilis cases	3.4 ± 1.1

A total of 21 blood samples from these 10 cases were tested. The results yielded by the WR and chol. WR for this Kahn-positive material are presented in Table 53.

Table 53.

The Number of Positive Results Yielded by the WR and Chol. WR for the Investigated Kahn-positive Alcoholic Material

	WR	Chol. WR	Kahn
Positive	3	7	21
Negative	18	14	—
Total	21	21	21

If the percentage illustrating the incidence of seropositive syphilis in the alcoholic material — 3.4 ± 1.1 — is compared with the corresponding percentage for pregnant women — 0.66 ± 0.05, the difference of the percentages 2.7 ± 1.1 is not statistically definite.

In connection with the prisoner material it emerged that the offences against the Alcoholic Beverages Act were more frequently represented in the Kahn-positive prisoner material than in the prisoner material covering the whole country. In that connection mention was made of DAHLBERG's (1949) studies, according to which the venereal disease frequency was higher among those sentenced for drunkenness than in the control material. He holds the opinion that the venereal diseases and alcoholism are both affected by a third factor — economic conditions. In the Canadian armed forces the venereal disease frequency among men who drank excessively was 19 times as great during the war as in the control material (WATTS and WILSON 1945). WITTKOWER (1948) mentions that among the venereal disease patients in the British armed forces there are 15 times as many soldiers who drink heavily as in the normal soldier material. In the U.S. army in Italy during the war time the corresponding ratio was 4 (BRODY 1948). In the restaurants in Helsinki, no correlation could be shown to exist between the quantities of alcohol served and the syphilis incidence of the restaurant personnel (PÄTIÄLÄ and TALVITIE 1951, PÄTIÄLÄ 1951).

The Incidence of Seropositive Syphilis in Poor Law Institute Dependents

The author's observations concerning the Poor Law Institute dependents are based on the material investigated in the Department of Bacteriology and Serology of the University of Turku during the period January 1, 1950 — August 16, 1951, comprising in all 502 serologically investigated persons. All the persons are from the Poor Law Institute of the town of Turku. Their ages vary from 30 to 96 years, the majority being over 70 years of age. During the last 15 years the average length of life of the female dependents has been 74 years 1 month 3 days, and that of the male dependents 73 years 11 months 2 days (OLLINEN, in press). The material was investigated in the way explained in connection with the material covering pregnant women and prisoners. Efforts have been made to exclude false positive results by means of inquiries. The incidence of seropositive syphilis in the investigated material covering Poor Law Institute dependents, in which the average age is over 70 years, is illustrated by the following Table:

Serologically investigated Poor Law Institute dependents ..	502
Seropositive syphilis cases	35
Percentage of seropositive syphilis cases	7.0 ± 1.1

A total of 62 samples from these 35 Kahn-positive dependents were investigated. The results yielded by the WR and chol. WR for this material are to be seen in Table 54.

Table 54.

The Number of Positive Results Yielded by the WR and Chol. WR for the Investigated Kahn-positive Material Covering Poor Law Institute Dependents Material

	WR	Chol. WR	Kahn
Positive	14	28	62
Negative	48	34	—
Total	62	62	62

When the percentage of seropositive syphilis frequency of the investigated material covering Poor Law Institute dependents (7.0 ± 1.1) is compared with the corresponding percentage of the material of pregnant women (0.66 ± 0.05), the difference between the percentages is manifest (6.3 ± 1.1). When evaluating the material one must take into consideration that the Poor Law Institute concerned is in a town with a population of 100,000. The Poor Law Institute being a social welfare institute, the dependents generally come from poor homes. On the basis of the material no generalization can be made with regard to the Poor Law Institute material of the whole country, which in the year 1946 comprised 13,804 dependents over 65 (MUSTALA 1949). In 1945 there were in the Poor Law Institutes of Finland 26,200 dependents and of them ca. $18{,}000 \geqq 60$ years of age (TUNKELO 1949). On the basis of the present material it is thus obvious that at least in the town of Turku among asocial old people seropositive syphilis is more general than the average syphilis frequency which the material of pregnant women deriving from the whole County of Turku and Pori and from all classes of society is considered to represent. From a material of this kind consisting of old people the "new" syphilis cases are certainly derived, which is why the percentage of those over

50 years in the reported syphilis material is on the increase as has emerged already in a previous connection.

THEWLIS and GALE (1948) are of the opinion that behind the hemiplegias, cerebral thromboses, dementia senilis and heart symptoms of the aged there might be unsuspected syphilis. They describe old people whose peptic troubles, pleurites, ischiae troubles, prostate troubles, eczemas, impaired hearing, cough, coronary troubles, leg ulcers, etc. were cured by antisyphilitic treatment, on which the suspected syphilis was confirmed. They are of the opinion that it is worth while to treat even the syphilis of the aged, as the treatment often brings relief from their various ailments.

DISCUSSION OF THE RESULTS CONCERNING THE SYPHILIS INCIDENCE

When comparing the territorial distribution of the new syphilis cases reported in the County of Turku and Pori in 1947 — 50 and of the seropositive syphilis material of the University of Turku with the territorial distribution of the syphilis cases treated in hospitals in the years 1877 — 1880 and derived from the County of Turku and Pori and of the gonorrhea cases reported in this county in years 1947 — 50, the attention was first attracted by the fact that over 70 years no great changes had taken place with regard to the relative territorial distribution of syphilis cases; quantitatively evaluated the average frequency of the known syphilis cases was in the years 1887 — 1880 about four times bigger than in the years 1947 — 50. The fact that no greater changes have occurred in the territorial distribution of syphilis cases over 70 years may perhaps be ascribed to the economico-geographical structure of the county, i.e. the most important economic centres, traffic routes, the occupational distribution of the population etc. have also remained largely unchanged during this time. There were hardly any differences in the totals of the new syphilis cases reported in 1947 — 50 and of the cases included in the seropositive syphilis material of the Department of Bacteriology and Serology of the University of Turku; both totals refer to the whole county. For different localities, on the other hand, a great deal of dissimilarity existed; most often there were more seropositive cases. This

has been attributed to the fact old, already reported syphilis patients have moved from one place to another.

The territorial distribution of the gonorrhea and syphilis cases also followed the same general lines, in other words, where there was more syphilis, there was also more gonorrhea. However, especially in the countryside numerous exceptions were found in this respect sometimes in one way, sometimes in the other. Judging by the present materials a higher proportion of the gonorrhea cases than of the syphilis cases derived from towns and market towns (difference between the percentages was 13.7 ± 1.3). A factor contributory to this difference is probably that all the towns of the county are harbour towns, and gonorrhea in Finland is to a great extent "imported goods", a fact illustrated above from earlier studies.

The distribution of seropositive syphilis cases into different syphilis stages was studied on the basis of a material of 818 cases. There were 2.4 % primary syphilis cases, 58.1 % secondary and latent syphilis cases, 8.7 % tertiary syphilis and neurosyphilis cases, and 5.7 % congenital syphilis cases; in 25.1 % of the cases the official physician concerned had not been able to decide which stage the syphilis had reached. As compared with the situation 70 years ago, a change had taken place at least in so far as the proportion of tertiary syphilis cases had decreased.

Regarding the percentual occupational distribution of the new syphilis cases reported in 1947 — 50, no statistically definite changes had occurred during the different years of the period of the time under examination. When considering the whole material for these years it must be regarded as fairly certain that the percentage of the agricultural population in the reported syphilis cases was smaller than its percentage of the population of the whole district under examination; for those without trade or occupation the opposite is true. As it is impossible, even tentatively, to estimate the percentages of the other trade groups of the population, the census statistics not yet having been completed, it could not be estimated to what extent their percentage of the reported syphilis cases corresponded to their proportion of the total population.

When examining the distribution of the new syphilis cases reported in the years 1947 — 50 by age groups, a statistically definite increase in the percentage of those aged $\geqq 50$ years could

be observed during the time under review, in the percentage of those aged 15 — 19 years a statistically definite decrease had taken place during the same period of time. With regard to other age categories no statistically definite changes could be observed; however, within the limits of statistical potential error, increase in the proportion of those aged 30 — 49, and decrease in the proportion of those aged 20 — 29 could be observed. This increase in the proportion of the older age categories is assumed to be due to decrease of syphilis, which leads to the result that the detected cases increasingly represent old cases not diagnosed before.

The incidence of seropositive syphilis in the material of ca. 26,000 pregnant women was 0.66 ± 0.05 %. The average birth-weight of the children of mothers suffering from seropositive syphilis was equal to that in the control material. Of the 96 mothers suffering from seropositive syphilis 89 were known to have had antisyphilitic treatment either during the pregnancy or earlier. This must be taken into consideration when evaluating the average birth-weight of the children. 3 of these 96 mothers were delivered of a stillbirth. According to calculations the 174 mothers included in the present material of pregnant seropositive syphilities had earlier had 54 abortions, while a non-syphilitic material of equal size would only have had 9 abortions. In the distribution according to sex of the children of mothers suffering from seropositive syphilis there was no definite difference, judged on the basis of the present material, as compared with the control material, consisting of the total material of live births in Finland in 1949. A statistically definite difference existed in the distribution according to sex of children born living or dead in Finland in 1949, i.e. there were more boys among still births than among live births. Within the limits of statistical potential error a similar difference was visible also in the distribution according to sex of the children of mothers suffering from seropositive syphilis and the children of mothers included in the control material.

The seropositive syphilis frequency in the prisoner material of ca 4,000 prisoners was 4.0 ± 0.3 %. In the Kahn-positive prisoner material there was a smaller proportion of first offenders than in the control material, which consisted of the total material of those committed to imprisonment or hard labour in Finland in 1947 (difference between the percentages was 20.0 ± 2.8). The criminal

registers of the Kahn-positive prisoners showed fewer crimes against state or community and more crimes against the Alcoholic Beverages Act than the control material, which consisted of the total prisoner material in Finland in 1949. From the statistical point of view there were on the whole an equal quantity of crimes against individuals and against property in both of the materials.

The incidence of seropositive syphilis in alcoholics was 3.4 ± 1.1 %, on the basis of the present material covering 295 alcoholics; thus no statistically definite difference could be found as compared with the material of pregnant women.

In the material comprising Poor Law Institute dependents (502 investigated) the seropositive syphilis frequency was 7.0 ± 1.1 %. The material consisted mostly of people over 70 years of age; the people concerned came primarily from poor economic circumstances, and a large number of them were crippled by various diseases. From among old people of this kind many of the "new" syphilis cases originate, which contribute towards raising the percentage of the older age categories of the reported syphilis material.

V.

The disease frequency of seropositive syphilis material, based on hospital patients.

SURVEY OF THE LITERATURE

It is difficult to find in the literature studies concerning the diseases of syphilitics. This is perhaps due to the fact that when syphilis has once been diagnosed in the patient, his later illnesses are often considered syphilitic. The American Social Hygiene Association has calculated that in the U.S.A. the proportion of acquired and congenital syphilis in the total mortality rate is 16 %. This figure is based on the calculation that in the cases of death caused by the diseases listed below the percentages given against the disease must be counted as caused by syphilis:

Tabes dorsalis	100 %
Progressive Paralysis	100 %
Congenital weakness, icterus and sclerema	100 %
Organic heart disease	50 %
Angina pectoris	50 %
Arterial diseases, sclerosis, aneurysm etc.	40 %
Cerebral hemorrhage, apoplexy	40 %
Softening of the brain	40 %
Chronic nephritis	20 %
Epilepsy	10 %
Encephalitis	10 %
Meningitis (in general)	10 %

SIMON (1948 *b*) says that in order to name all the diseases which have been considered to be syphilitic "one should go through the whole pathology". As examples he lists, in addition to the known syphilis diseases, the following diseases, which have been ascribed

to syphilis: hypertension, hypotension, paroxysmal tachycardia, angina pectoris, phlebitis, ulcus varicosum, purpura dermatitis, Raynard's disease, hysteria, neurasthenia, epilepsy, hemiplegia, facial paralysis, facial neuralgia, spastic paraplegia, radiculitis, syndroma lumbosacrale; he mentions that in the literature even syphilitic appendicitis is spoken of. SIMON also refers to certain statistics according to which 90 out of 100 deaths from heart disease are due to syphilis and out of every 100 people with nervous diseases the cause can be tracted back to syphilis in 30 cases. It is certainly true that it is difficult to decide for each separate case whether some disease in a syphilitic is syphilitic in origin or not. Not all diseases lead to the autopsy table, and even the help given by the autopsy findings is often questionable. It may happen that in the autopsy nothing indicative of syphilis is found in a known seropositive syphilitic, and in seronegative cases the situation may be the reverse (BLACK and ROSAHN 1944). With the present antibiotic treatment the clinical evaluation of the situation has become still more difficult.

The present subject primarily calls for the following of the incidence of certain common diseases or pathological conditions in seropositive syphilitics. The literature on this subject is not very copious. However, the observations reported by DUMBAR (1945) should be mentioned here. He compared the venereal disease in patients suffering from various diseases and had observed that the venereal disease incidence was above the average in those suffering from coronary diseases, in hypertonics and tonsillitis patients; with diabetics the frequency was lower than average. Patients with rheumatic fever or rheumatoid arthritis had an average venereal incidence. A higher than normal incidence also appeared in patients who were hospitalized for spontaneous or induced abortion.

SMITH, SAXTON and FRITZ (1951) investigated the autopsy findings of 398 persons who had suffered from cardiovascular syphilis and of 459 persons with rheumatic heart trouble, and found both were present in 8 cases. They mention that in the literature 47 syphilitic cardiovascular diseases combined with chronic endocardial disease are listed. They calculate the incidence of this kind of combination to be 1 — 10 % in the autopsy material.

Cancer and syphilis are often mentioned in connection with each other. The theory has been advanced that the syphilis toxins

weaken the organism, and thus predispose the formation of carcinoma (STRANDBERG 1921). STRANDBERG also quotes the surgeon v. ESMARCH: "The rodent ulcer is in many cases nothing other than a form of lues inveterata." In syphilitic rabbits cutaneous carcinoma could be produced more quickly than in control animals (CASTIGLIONI 1933, TRUFFI and CERUTTI 1936). The results of these investigators could not be supported by BESSEMANNS and MAISIN (1942), who used mice as test animals, nor by RØJEL (1948), who used rabbits. Seldom does the syphilitic process in the skin turn into carcinoma, and LEWANDOWSKY (1916) says that epithelioma on the skin after lupus vulgaris is much commoner than after a syphilitic process. In Finland LAITINEN (1945) examined 2,073 cancer patients and found syphilis in 6.6 % of them, he computed the average syphilis frequency of the Finnish population of cancer age to be 1 — 2 %, and reached the opinion that syphilis patients are more disposed to cancer than non-syphilitic persons.

FOUCAR and STOKES (1921) examined 4,800 syphilities and found pernicious anaemia in 25 of them. In WILKINSON's (1933) material comprising 370 pernicious anaemia patients there were 6 who also had syphilis. ZIVIN and LEROY (1949) describe 3 such combinations, and in their cases they did not observe any causal relation between the diseases.

RÁVNAY (1949) describes 36 known lymphogranuloma inguinale patients from a clinic in Szeged. Syphilis was also found in 15 of them. In 5 of these cases there was a dual infection, but in 8 cases syphilis had been the first infection and in 2 cases lymphogranuloma inguinale had been the first. The author advances the theory that syphilis patients may be more exposed to lymphogranuloma inguinale infection than non-syphilities.

ESKOLA (1948) analysed the autopsy material of the Pathologico-Anatomic Institute of the University of Helsinki and arrived at the conclusion that syphilis infection to some extent contributes towards the development of arteriosclerosis.

STOKES, BEERMAN and INGRAHAM (1944) in their report on manual studies suggest that syphilis patients may be disposed to achlorhydria or hypoacidity.

PRESENT OBSERVATIONS OF THE DISEASE RATE OF SEROPOSITIVE SYPHILITICS

Material and Method

The present observations are based on a material derived from the materials of seropositive syphilities in the serologic part of this study. In this material are included the patients who have been tested in the Medical, Surgical, Ophthalmologic, Gynecologic or Oto-rhino-laryngologic Clinics of the University of Turku. The distribution of the material between these different clinics can be seen from the following:

Medical Clinic of the University of Turku	350 cases
Surgical „ „ „ „ „ „	224 „
Ophthalmologic Clinic of the University of Turku	77 „
Gynecologic „ „ „ „ „ „	53 „
Oto-rhino-laryngologic Clinic of the University of Turku	28 „
Total	732 cases

Comparisons have been made with regard to some most common diseases, and as control material the nonsyphilitic material of the corresponding Turku University clinics for the years 1946 — 50 has been used. The cases are discussed by clinics, except for peptic ulcers and carcinomas, which are presented separately.

Internal Diseases

350 seropositive syphilitics of the present material were treated by the medical wards. The largest group consisted of syphilitic affections of the aorta, a total of 84 cases. A total of 89 patients in the material of seropositive syphilitics treated in these wards proved to be cases treated for syphilis alone. These, naturally, must be excluded, and hence the material will cover 261 patients.

The seronegative control material totals 16,472 cases. It is necessary to exclude the 1,653 patients whose disease was not present at all in the patients of the material investigated. Hence the seronegative control material proper will consists of 14,819 patients.

The calculations on pneumonia, kidney diseases, hepatitis, hepatic cirrhosis and apoplexy are compiled in Table 55 below.

Table 55.

Calculation of the Incidence of Certain Internal Diseases in Seropositive Syphilitic Hospital Patients, as Compared with the Control Material

	Seropositive syphilis cases	Total cases	Percentage of seropositive syphilitics	Deviation of the percentage of seropositive syphilitics from computed percentage
Total material .	261	15,080	1.7 ± 0.1	(1.7 ± 0.1 = computed percentage)
Pneumonia	24	1,073	2.2 ± 0.3	$+ 0.5 \pm 0.3$
Kidney diseases.	20	740	2.7 ± 0.6	$+ 1.0 \pm 0.6$
Hepatitis	9	269	3.3 ± 1.0	$+ 1.6 \pm 1.0$
Hepatic cirrhosis	8	67	11.9 ± 4.0	$+ 10.2 \pm 4.0$
Apoplexy	15	440	3.4 ± 0.9	$+ 1.7 \pm 0.9$

Pneumonia: In both groups of patients pneumonia was equally frequent.

In one case the diagnosis arrived at was clinical pulmonary syphilis; this case is not included in the above cases of pneumonia.

In 3 cases pneumonia appeared together with syphilitic affection of the aorta and in 1 case, in addition to pneumonia, cardiac insufficiency due to nonsyphilitic causes was involved.

Regarding the incidence of true pulmonary syphilis BEERMAN, STOKES and INGRAHAM (1944) mention as an example the studies of OSLER. He found lung syphilis in 12 cases from an autopsy material comprising 2,500 autopsied patients suffering from acquired syphilis.

Kidney Diseases: The cases of nephritis, nephrosis, pyelonephritis, pyelitis and pyelocystitis treated in the wards were included, and a comparison was made between the seropositive syphilis patients and nonsyphilitic patients.

The deviation from the calculated value given in Table 55 is insignificant. Accordingly, there were in both patient groups an equal number of kidney diseases within the limits of statistical potential error.

In 5 cases there appeared in the seropositive syphilis a disease picture of the nephrosis type, in 2 cases the kidney lesion led to uraemia. In 2 cases the patient had a syphilitic affection of the aorta in addition to the kidney lesion.

KARVONEN (1901) on the basis of an autopsy material concluded that in 25 — 50 % of syphilitics the kidneys are specifically affected. THOMAS and SCHUR (1946) are of the opinion that 1 % of secondary syphilitics have syphilitic nephrosis, the only clinical symptom of which is slight albuminuria. FURMAN, GALE, ORY and WEINSTEIN (1951) examined a negro, with such a disease picture, using p-aminohippuric acid and inulin clearance. These tests proved that the tubules and glomerules were equally injured in this kind of slight syphilitic nephrosis. Penicillin treatment fully cured the condition.

The present material also included two paroxysmal cold hemoglobinuria cases with positive seroreactions for syphilis. When reporting the history of paroxysmal cold hemoglobinuria MACKENZIE (1926) says that MURRI (1880) even then connected the disease with syphilis. BECKER (1948) is of the opinion that if the patient has paroxysmal hemoglobinuria and positive seroreactions for syphilis, then syphilis is always in question, even though no other syphilis symptoms can be found.

Hepatitis and Liver Cirrhosis: The deviation from the calculated value concerning hepatitis presented in Table 55 is insignificant.

In 2 cases of hepatitis of seropositive syphilitics the hepatitis has been clinically considered as "salvarsanhepatitis", the rest were ordinary cases of acute hepatitis.

ALSTED (1947) in Denmark examined 54 malignant hepatitis patients, of whom 27 died of the disease. None of them had any history of syphilis infection or arsenic therapy.

The deviation concerning liver cirrhosis presented in Table 55 is statistically probable. In 1 liver cirrhosis case of a seropositive syphilitic, affection of the aorta was also concerned.

HALONEN and SAKSELA (1946) went through the autopsy material of the Pathologico-Anatomic Institute of the University of Helsinki, Kivelä Hospital and Maria Hospital, and found 134 liver cirrhosis cases or 1.8 % of the total investigated material. Regarding 100 of these autopsied cirrhosis patients sufficient anamnestic

information was available, and among them there were 19 who had had syphilis.

Apoplexy: All the patients of the medical wards with a diagnosis of intracranial haemorrhage, embolia cerebri, thrombosis cerebri, hemiplegia or merely apoplexy cerebri were included.

The deviation presented in Table 55 must be considered as insignificant.

No other diseases belonging to the medical section will be discussed, partly because of the limited number of the cases and partly because of difficulties in interpretation. For many diseases it is difficult to estimate the date of onset of the disease, and thus the chronological relationship of the disease concerned and the syphilis infection remains uncertain; such an uncertainty is present in a number of the kidney-disease cases mentioned.

Surgical Diseases

On surgical wards 224 patients included in the present material of seropositive syphilities were treated. Only the cases of appendicitis, fractures, concussion and contusion of the brain, and the cases of hypertrophy of the prostate will be discussed. It must be presumed that the hypertrophy of the prostate cases caught their syphilis infections before the prostate trouble began to manifest itself; this chronological relation cannot be stated as certain.

Eight of the seropositive syphilities treated by the Surgical Wards were hospitalized for syphilis alone. The material to be discussed will therefore consist of 216 seropositive syphilities treated for surgical diseases.

The seronegative control material covers a total of 25,144 patients. It is necessary to exclude from this total the 2,233 patients whose diseases were not represented in the material to be studied. Hence the total of the seronegative control material will be 22,911 surgical patients.

The calculations on the cases of appendicitis, fractures, concussion and contusion of the brain, and of hypertrophy of the prostate are given in Table 56.

Appendicitis: All the appendicitis cases nursed in the surgical wards during the years 1946 — 50 have been taken into consideration.

Table 56.

Calculation on the Incidence of Certain Surgical Diseases among Seropositive Syphilitic Hospital Patients as Compared with the Control Material

	Seropositive syphilis cases	Total cases	Percentage of seropositive cases	Deviation of the percentage of seropositive syphilities from computed value
Total material .	216	23,127	0.9 ± 0.1	(0.9 ± 0.1 = computed value)
Appendicitis ...	12	2,046	0.6 ± 0.2	-0.3 ± 0.2
Fractures	22	2,433	0.9 ± 0.2	$+0.0 \pm 0.2$
Commotio & contusio cerebri ..	20	1,344	1.5 ± 0.3	$+0.6 \pm 0.3$
Hypertrophia prostate	9	370	2.4 ± 0.8	$+1.5 \pm 0.8$

The deviation from computed value given in Table 56 is insignificant. The appendicitis frequency thus, within the limits of statistical potential error, was equally high in both of the patient groups.

Of the appendicites of seropositive syphilities presented 5 had led to perforation of the appendix before admission to the hospital (these patients were aged: 75 yrs, 50 yrs, 44 yrs, 43 yrs, and 25 yrs) and in 3 cases gangraenous appendicitis had occurred; in 4 cases the inflammation was slight in character. In the control material the perforation incidence was 29.7 ± 1.0 %. In RENKONEN's (1946) appendicitis material covering the whole of Finland the perforation incidence was 16.9 %.

Fractures: All the fractures from the years 1946 — 50 treated in the hospital were collected. The fractures treated at the Out-Patients' Clinic have not been taken into consideration.

There were, as we can see from Table 56, an equal number of fractures in both patient groups.

Table 57 presents the proportion of collum femoris fractures of the fractures of the both patient groups under review.

The difference between the percentages presented in Table 57 is statistically probable.

Table 57.

The Proportion of Collum Femoris Fractures of the Fractures of the Seropositive Syphilities as Compared with the Nonsyphilitic Material

	Seropositive syphilities	Non-syphilities
Fractures in total	22	2,411
Collum Femoris Fractures included	8	266
Percentage of the Collum Femoris Fractures ..	36 ± 10	9.4 ± 0.6
Difference between the percentages	27 ± 10	

In PUTTI's (1942) material, which comprised 7,994 fractures patients, collum femoris fracture occurred in 828 (10.35 %). 52.35 % of these collum femoris fracture patients were over 60 years of age. In consulting a number of books on fractures (MAGNUSON 1942, SPEED 1942, WATSON-JONES 1946) the author did not observe that any attention had been paid to the possible extent in which the fractures of syphilities might include a larger part of collum femoris fractures than the control material. WATSON-JONES (1946) emphasizes, it is true, the disposition of tabetics to fractures and the fact that syphilitic ostites make the bones fragile, so that fractures can more easily occur. MYHRE (1951) has described a woman of 64, who had positive seroreactions for syphilis and sciatic pains on one side. In the joint concerned sclerotic ostitis was diagnosed, which MYHRE interpreted to be syphilitic. The probable difference appearing in the material the author would attribute primarily to the fact discussed previously, that the proportion of older age categories among the syphilities has increased, and thus in the syphilitic material more fractures of the aged appear.

Commotio and Contusio Cerebri: The control material is the total number of patients of the Surgical Clinics of the University of Turku for the years 1946 — 48 includes commotio and contusio cerebri cases.

The deviation from the calculated value presented in Table 56 is statistically probable.

Trauma might activate syphilis of latent stage. STEFANI (1949) describes cases in which general paralysis has started to develop after a trauma. PANCONESI (1949) reports on a 22-year-old soldier,

who might have 2 years earlier caught a syphilis infection, which, however, had not been observed. He met with a traffic accident upon which the seroreactions became positive and the patient developed hyperkeratotic nodules on his hands; specific antisyphilitic treatment remedied the condition.

Hypertrophia prostatae: The deviation from the calculated value presented in Table 56 is statistically insignificant.

It is not considered relevant to discuss the other surgical diseases.

Peptic Ulcer

The gastric and duodenal ulcers treated in 1946 — 50 in the medical and surgical wards were included. The results of the comparison are presented in Table 58.

Table 58.

The Proportion of Peptic Ulcer Patients among the Seropositive Syphilitic Patients of the Medical and Surgical Wards as Compared with Nonsyphilitic Material

	Seropositive syphilitics	Non-syphilitics
Peptic Ulcer Patients	26	1,579
Medical and Surgical Patients, total	477	37,720
Incidence of Peptic Ulcer Patients (%)	5.5 ± 1.0	4.2 ± 0.1
Difference between the percentages	1.3 ± 1.0	

The percentages of Table 58 correspond. In both patient groups, accordingly, an equal number of peptic ulcer patients appeared.

Of the peptic ulcer patients among the seropositive syphilitics in the material, 4 were admitted to the hospital on account of haematemesis, in 2 cases the ulcer had perforated before arrival in the hospital.

There is some uncertainty in the material with regard to the relative dates of onset of the syphilis infection and the peptic ulcer.

The study of KURTHI (1948) deals with the incidence of the true syphilitic peptic ulcer. In Hungary over a period of 16 years he had seen 3,066 peptic ulcer patients; 52 cases (1.7 %) were of

syphilitic peptic ulcer. STOKES, BEERMAN and INGRAHAM (1944) mention that MOUTIER had examined 8,000 persons suffering from diseases of the alimentary tract and found in this material 8 cases of abdominal syphilis. CLASEN (1930) is of the opinion that haemorrhage from syphilitic peptic ulcer is more rare than from ordinary peptic ulcer.

Carcinoma

The cancer cases were derived from the medical and surgical wards and oto-, rhino- and laryngologic diseases ward. The comparison is given in Table 59.

Table 59.

The Proportion of Carcinoma Patients among the Seropositive Syphilitic and the Nonsyphilitic Patients of the Medical and Surgical Wards and Oto-, Rhino- and Laryngological Diseases Ward

	Seropositive syphilities	Non-syphilities
Carcinoma Patients	18	1,308
Patients, total	505	42,380
Incidence of the Carcinoma Patients	3.6 ± 0.8	3.1 ± 0.1
Difference between the percentages	0.5 ± 0.8	

The percentages of Table 59 correspond. In both patient groups, accordingly, an equal number of carcinoma patients appeared.

The following list gives some idea of the carcinoma localizations of the seropositive syphilis material:

Carcinoma	mammae	4 cases
„	labii	2 „
„	oesophagi	2 „
„	laryngis	2 „
„	ani	1 „
„	org. varior.	7 „

In Table 60 there is a comparison between the cases of the carcinoma labii, oesophagi, laryngis and ani in both of the patient groups.

Table 60.

The Proportion of the Carcinoma labii, oesophagi, laryngis and ani Cases among the Carcinoma Cases of the Seropositive Syphilitic and the Nonsyphilitic Material

	Seropositive syphilities	Non-syphilities
Carcinoma labii, oesophagi, laryngis and ani cases, total	7	151
Carcinoma Cases, total	18	1,308
Proportion of the above Carcinoma Cases of the Total Carcinoma Material (%)	39 ± 11	11.5 ± 0.9
Difference between the percentages	27 ± 11	

The difference between the percentages given in Table 60 is statistically satisfactory.

In LAITINEN's (1945) material mentioned earlier, there were greatest number of syphilis cases among women suffering from carcinoma of the genitals (in Table 59 and 60 gynecologic carcinoma cases are not included); the next in order were those suffering from carcinoma of active respiratory organs, those suffering from the carcinoma of tongue and mouth, rectum and anus, and those suffering from the carcinoma of the basal cells of the skin or of the lips.

Gynecologic Diseases

The material includes 53 patients from the gynecologic wards. 29 of them had inflammatory diseases of women, 13 abortion, 13 myoma, 3 carcinoma uteri, etc. (the same patient may have had several diseases).

The seronegative control material covers 5,309 patients. For the reason discussed before 390 patients must be excluded, and hence the seronegative control material will total 4.919 patients.

Only the cases of myoma uteri and abscessus fossae Douglasi will be discussed here. The calculations on these are given in Table 61.

Myoma uteri: The deviation given in Table 61 is statistically satisfactory.

Table 61.

Calculation of the Proportion of the Cases of Myoma uteri and Abscessus fossae Douglasi among the Seropositive Syphilitic Gynecological Patient Material as Compared with the Control Material

	Seropositive syphilities	Total cases	Percentage of seropositive syphilities	Deviation of the percentage of seropositive syphilities from computed value
Total material ..	53	4,972	1.1 ± 0.2	(1.1 ± 0.2 = computed value)
Myoma uteri	13	578	2.3 ± 0.6	$+ 1.2 \pm 0.6$
Abscessus fossae Douglasi	6	57	11 ± 4	$+10 \pm 4$

The chronological relationship of the dates of onest of the syphilities infection and myoma uteri in the material is uncertain.

VEIT and STOECKEL (1931), in their manual, doubt whether syphilis plays any etiological part in the origin of myoma. In the autopsy material of KELLY and CULLEN (1909) the myoma was three times more frequent among the negroes than among the white women of the U.S. As pointed out previously, practically every third negro in the U.S.A. has syphilis.

Abscessus fossae Douglasi: The deviation presented in Table 61 is statistically probable.

In seeking explanation of the fact that a probably larger proportion of syphilis patients than of the patients included in the control material are abscessus fossae Douglasi patients, the social factors must certainly be taken into consideration. The majority of the abscessus fossae Douglasi patients come from social categories (various degrees of vagrancy, etc.) in which the syphilis prevalence is higher than average.

Ear Diseases

The material includes 28 seropositive syphilities, treated on the ward of oto-rhino-laryngologic diseases. 2 of these had carcinoma of the larynx and 2 carcinoma of the oesophagus. 10 had purulent inflammation of the tympanum or the nasal sinuses. The rest were "aseptic" operation patients.

Eye Diseases

On the ward of ophthalmologic diseases 77 patients of the material were tested. The following list gives the most important of the diseases for which the syphilitics were treated.

Keratitis parenchymatosa e syphilis congenita	27	cases
Neuritis et atrophia nervi optici	5	„
Iritis	5	„
Cataracta senilis	4	„
Glaucoma	4	„
Dacryocystitis purulenta	3	„
Morb. varior	29	„

The comparatively large number of the Keratitis parenchymatosa patients perhaps is due to the fact that the ward concerned was included in the material for a doctorial thesis on this disease and patients were gathered there. Nothing in the character of the material indicated anything deviating from the normal in distribution of the patients between the different diseases, if specific diseases are excluded.

DISCUSSION OF THE RESULTS CONCERNING THE DISEASE RATE OF SEROPOSITIVE SYPHILITICS

When examining the results concerning the disease rate of seropositive syphilities it must be taken into consideration that hospital material only is concerned. In addition it must be remembered that the only guarantee for the non-syphilitic nature of the control material is its seronegativity. Thus seronegative syphilities might be included in the control materials. However, the control materials are so extensive that these cases are probably of no significance.

When considering the results of the comparisons the attention is attracted by the fact that for no disease reviewed could a marked difference be found between the seropositive syphilitic hospital patients and the control material. Thus the following diseases appear in the limits of statistical potential error in equally large numbers among both materials: pneumonias, kidney lesions, hepatitis, apoplexy, appendicitis, fractures, hypertrophy of the prostate, peptic

ulcer, and carcinoma. In the seropositive syphilitic hospital material the following diseases appeared in a statistically *satisfactorily* larger extent than in the control material: commotio et contusio cerebri and myoma uteri. Liver cirrhosis patients were statistically probably more numerous in the seropositive syphilitic internal disease material than in the corresponding control material. The proportion of the abscessus fossae Douglasi patients among the seropositive syphilitic gynecologic patients was statistically *probably* larger than in the control material. The proportion of the collum femoris fractures of the fractures of the seropositive syphilities was *probably* larger than in the control material. The proportion of carcinoma labii, oesophagi, laryngis and ani cases among the carcinoma cases of the seropositive syphilis was statistically *satisfactorily* larger than in the control material.

Excluding the small exceptions without full statistical certainty, the present seropositive syphilitic hospital material can be taken as agreeing with the control material with regard to the incidence in both groups of the diseases considered.

VI.

Summary

The present investigation is based on the following serologic materials:

— The material of 126,344 blood samples tested in the Department of Bacteriology and Serology of the University of Turku in 1946 — 50; on each specimen the Kahn reaction has been performed, and on 79,719 specimens the Wassermann reaction and Wassermann reaction sensitized with cholesterol in addition.

— The material of 7,928 samples tested in 1951; on each specimen the above mentioned three routine reactions were made and in addition two cardiolipin slide tests (VDRL and Rein — Bossak) and one sitolipin slide test (with VDRL technique); on 4,260 specimen Meinicke's clarification test and on 4,414 specimens the Berger slide test were also performed; all eight reactions were made on 3,014 specimens.

— The material of 5,116 samples tested in 1951; on each specimen, in addition to the routine reactions — Wassermann, Wassermann sensitized with cholesterol, Kahn — the Wassermann reaction with the cardiolipin and sitolipin antigens was performed, with Wassermann reaction carried out with these 4 different antigens 238 cerebrospinal fluid samples were also tested.

The seropositive syphilis material deriving from these serologic materials was subjected to three examinations of different kinds, and for this reason the present study is divided into three principal sections. In the serologic section various seroreactions and comparisons concerning the syphilis antigens are made with special reference to the position of the cardiolipin and sitolipin antigens as syphilis antigens. The section dealing with the incidence of seropositive syphilis on the basis of the present seropositive syphilis material reviews the territorial distribution of seropositive syphilis

cases in the operation territory of the Department of Bacteriology and Serology of the University of Turku, covering the County of Turku and Pori (an area of 22,000 sq. km with 611,000 inhabitants); comparisons are made with the reported syphilis material, gonorrhea material and the syphilis material for 1877 — 80. In this section observations are also made on the incidence of seropositive syphilis in the material of pregnant women (ca. 26,000 pregnant women examined), in the prisoner material (ca. 4,000 prisoners examined), in the alcoholic material (295 alcoholics examined) and in the material covering Poor Law Institute dependents (502 examined). The third and last section of the study continues to review the patients included in the same seropositive syphilis material, which patients have been treated in the wards of internal, surgical, gynecologic, ophthalmologic and oto-rhino-laryngologic diseases (total 732 cases). In this section comparisons are made regarding the frequency of certain diseases in the seropositive syphilitic material and in the seronegative and thus probably mostly nonsyphilitic hospital material. In the beginning of each section of the study the literature connected with the subject is surveyed.

The Serological Section: On the basis of serologically investigated material for 1946 — 50 it could be stated that during these five years the proportion of the Wassermann-positive "new" syphilis cases of the seropositive material had decreased from 62.4 to 32.4 % and the proportion of the cholesterol Wassermann-positive cases from 78.9 to 53.7 %. The percentage of the Kahn-positive cases, however, had remained the same. This phenomenon the author considers to be due to the fact that fresh syphilis has simultaneously decreased considerably in Finland, so that the "new" syphilis cases entered in the books of the laboratory were by 1950 mostly old and treated syphilis cases. The course of the syphilis epidemics in Finland, the peak of which was reached in 1946 — 47, is clearly visible in the percentage of the Wassermann-positive cases. Weak positive results were obtained both for the Wassermann, cholesterol-Wassermann and Kahn reactions, with an equal number of results, on the whole, for each of them (7.5 — 9.4 %). For this reason the author assumes that the number of weak positive results does not depend on the syphilis antigen used or the technique used, but that this margin of weak positive results always remains largely the same.

When comparisons were made between the serologic results obtained with the cardiolipin and sitolipin antigens, the attention was attracted throughout by the similarity of the results obtained with them. When used as flocculation antigens, no significant differences appeared in the percentages illustrating the average sensitivity of the cardiolipin and sitolipin antigens in different groups of syphilis cases (Tables 11 — 14), in the group of patients whom antisyphilitic treatment of different stages had been given, the percentages illustrating the positive results obtained with the cardiolipin and sitolipin antigens are also statistically equally large (Tables 17 — 21), and the differences between the cardiolipin and sitolipin antigens appearing in the curves illustrating the results obtained for quantitatively performed flocculation reactions must be considered as insignificant in practice (Fig. 3). An equal quantity of false positive flocculation reaction results were obtained with both antigens (Table 26). When employing the cardiolipin and sitolipin antigens in the complement fixation reaction (Wassermann) with both antigens an equal number of positive results were obtained, and the same with the false positive results (Tables 32 and 36). In the quantitative complement fixation reaction on the other hand the positivity obtained for sitolipin antigen tended to lag one or two dilution degrees behind the positivity obtained for the cardiolipin antigen. In the investigation series concerning the antigens of the complement fixation reaction the Wassermann reaction carried out with the cardiolipin and sitolipin antigens yielded similar results in 99.3 % and a differing result in 0.7 %. On the basis of all these results the author considers the cardiolipin and sitolipin antigens to be of equal value both in sensitivity and specificity. Their serologic relationship is at least as close as the similarity of their chemical structure.

When the results obtained for the Kahn reaction were compared with the results obtained for the cardiolipin and sitolipin slide tests, it was found that the Kahn reaction gave an equal number of or rather more positive results in definite syphilis cases than the cardiolipin and sitolipin slide tests; on the other hand the Kahn reaction yielded more false positive results. The percentage of false positive results of the total of Kahn reactions performed was 2.2, for the cardiolipin slide tests it was 1.3 and for the sitolipin slide tests 1.4, (see Table 26 for details). The Kahn

reaction yielded more clearly positive results than the Wassermann reaction performed with the cardiolipin and sitolipin antigens (Table 32). When evaluating the serologic results obtained the author draws attention to the fact that the Kahn reaction was read using 6 × magnification, which certainly increased the number of the positive results obtained for it; thus the number of false positive results also increased. The cardiolipin and sitolipin slide tests were read using the same magnification.

Meinicke's clarification test yielded slightly more positive results for definite syphilis cases than the cardiolipin and sitolipin slide tests but on the other hand slightly less false positive results. In both cases, however, the differences fell within the limits of statistical potential error. The author is of the opinion that Meinicke's clarification test is entirely equal to the cardiolipin and sitolipin slide tests in the sensitivity and specificity of the reaction.

The Berger slide test performed according to the original method gave as many positive results for definite syphilis cases as the Kahn reaction on the whole, whereas the number of false positive results yielded by it was unsuitably high (4.1 %, see Table 26 for details). The author thinks that a shorter rotation time when performing the reaction might have decreased the number of false positive results; in the literature it is stated that it is so, although no comparative materials are presented.

An equal quantity of positive results was obtained for the Wassermann reaction in cerebrospinal fluid samples, irrespective of whether it was performed with raw beef heart antigen not sensitized with cholesterol, raw beef heart antigen to which 0.15 % of cholesterol was added, cardiolipin antigen or sitolipin antigen (Table 38).

The majority of the diagnoses of the patients for whom false positive results were obtained were various infectious diseases, particularly infections of the respiratory tract. In the material covering 26,432 pregnant sera the Kahn reaction yielded false positive results, technical errors included, in 0.43 %.

The Section Dealing with Syphilis Incidence: When comparing the territorial distribution of the syphilis cases in the County of Turku and Pori in the years 1947 — 50 and 1877 — 80, the attention was drawn to the fact that no major changes had taken place

during this period of 70 years. The author thinks this to be primarily due to the fact that during the time concerned the economico-geographical structure of the county (traffic routes, economic centres, etc.) had not undergone any major changes. Quantitatively evaluated the incidence of the known syphilis cases in 1877 — 80 was four times higher than for 1947 — 50. The territorial distribution of the gonorrhea and syphilis cases was also on the same lines, in other words: where there was more syphilis, there was also more gonorrhea. Especially in the countryside many exceptions to this general development were found. On the basis of the present materials it appears that a proportionally larger proportion of the gonorrhea cases than the syphilis cases were derived from towns and market towns (the difference between the percentages was 13.7 ± 1.3). This difference is presumed to be due to the fact that all the towns of the county are harbour towns. Gonorrhea is largely "imported goods" in Finland, a fact which is attested in the literature.

The distribution of the seropositive syphilis cases by different stages of syphilis has been shown on the basis of 818 cases. The were 2.4 % primary syphilis cases, 58.1 % secondary and latent syphilis cases, 8.7 % tertiary syphilis cases and 5.7 % congenital syphilis cases; for 25.1 % of the cases the physician concerned had not reported the stage of the disease. Compared with the syphilis situation 70 years earlier, the situation had changed at least in the respect that the proportion of tertiary syphilis cases had decreased.

With regard to the distribution by trade or occupation of the syphilis cases reported in 1947 — 50, no statistically definite changes had taken place during the different years of the period under review. On the basis of the total material for these years it must be considered evident that the proportion of the agricultural population in the reported syphilis cases is smaller than its proportion of the total population of the territory under examination; for those without any trade or occupation the reverse is true (Table 42 and Fig. 3.).

When examining the distribution of the syphilis cases reported in 1947 — 50, by different age categories, a statistically definite percentage increase in the proportion of those $\geqq 50$ years of age during the time under review could be observed; regarding the

proportion of those aged 15 — 19 years a statistically definite decrease had taken place during the same time. Within the limits of statistical potential error a small increase could also be observed in the percentage of those aged 30 — 49 years and decrease in the percentage of those aged 20 — 29 years (Table 43 and Fig. 4). This increase in the percentage of the older age categories is presumed to be due to the decrease of syphilis in Finland.

The incidence of seropositive syphilis in the material covering ca. 26,000 pregnant women was 0.66 ± 0.05 %. Of 96 pregnant women suffering from seropositive syphilis 89 had definitely been given antisyphilitic treatment either during or before pregnancy. The average birthweight of the children they were delivered of was the same as in the control material (Table 46). 3 of them had been delivered of a stillbirth (in one case the cause of the death was premature ablation of the placenta). According to the calculations the 174 mothers, included in the total material of pregnant seropositive syphilitics, had had 54 earlier abortions, while the number of earlier abortions of an equally large nonsyphilitic material was 9. Regarding the distribution by sex, there was no statistically definite difference between the children of mothers suffering from seropositive syphilis and the control material, which consisted of the total material of live births in Finland in 1949. A statistically definite difference could be ascertained between those born living and dead in the whole of Finland during the year 1949 in so far as there were more male children among the stillbirths. Within the limits of statistical potential error a similar difference also appeared with regard to the distribution according to the sex of the children born of seropositive syphilitics (Table 47).

The seropositive syphilis frequency in the prisoner material of ca. 4,000 prisoners was 4.0 ± 0.3 %. The Kahn-positive prisoner material consisted a lower percentage of first offenders than the control material (the difference between the percentages was 20.0 ± 2.8, see Table 50 for details). In the criminal registers of the Kahn-positive prisoners there appeared fewer crimes against state and community and more crimes against the Alcoholic Beverages Act than in the control material. There were, on the whole an equal number of crimes against individuals and property in both of the materials (Table 52).

The seropositivity frequency obtained for the material of 295 alcoholics was 3.4 ± 1.1 %. There appeared no statistically definite difference as compared with the material of pregnant women.

In the material comprising Poor Law Institute dependents (502 examined) the seropositive syphilis frequency was 7.0 ± 1.1 %. The material consisted mainly of persons over 70 years of age coming from poor economic circumstances; a large number of them suffered from various diseases. The author assumes that from among old people of this kind originate many of the "new" syphilis cases, which contribute to the increase in the percentage of the older age categories of the reported syphilis material.

The Section Dealing with the Disease Frequencies of Seropositive Syphilis Material Based on Hospital Patients:

Within the limits of statistical potential error the incidence of the following diseases in the seropositive syphilitic hospital patients and in the control material was the same: pneumonia, kidney diseases, hepatitis, cerebral apoplexy, appendicitis, fractures, hypertrophy of the prostate, peptic ulcer and carcinoma. In the seropositive syphilitic hospital patients there appeared a statistically satisfactorily greater amount of the following diseases than in the control material: commotio et contusio cerebri and myoma uteri. The proportion of the liver cirrhosis and the abscessus fossae Douglasi patients of the seropositive syphilitic internal disease and gynecologic patients was statistically probably larger than in the control material. The proportion of the collum femoris fractures of the fractures of the seropositive syphilities treated in hospitals was statistically probably larger than in the control material. The proportion of the carcinoma labii, oesophagi, laryngis and ani cases among the carcinoma cases of the seropositive syphilities in hospital was statistically satisfactorily larger than in the control material.

Except for small exceptions without full statistical significance the seropositive syphilitic hospital patient material under review can be considered as corresponding with the control material regarding the incidence of the diseases under review in both patient groups.

VII.

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